THE FUNCTIONS OF THE ENDOCRINE GLANDS

THE FUNCTIONS

OF THE

ENDOCRINE GLANDS

By

PETER F HALL M D M R C P M R A C P

Assistant Physician Sydney Hospital
Assistant Lecturer in Physiology University of Sydney

HORWITZ PUBLICATIONS INC Sydney
PITMAN MEDICAL PUBLISHING CO LTD London
W B SAUNDERS COMPANY Philadelphia

First Published February 1959
Reprinted May 1959

Horwitz Publications Inc
Sydney N S W Australia

SET IN INTERTYPE TIMES ROMAN
AUSTRALIAN BURNIE SUPER CALENDERED PAPER
PRINTED IN AUSTRALIA BY HALSTEAD PRESS SYDNEY
Registered in Australia for transmission through the post as a book

To P M F BISHOP
whose generous encouragement
provided the stimulus for writing this book

PREFACE

To write a book about the endocrine glands is to aim at a moving target between writing and publication it is probable that some part of such a book will become out of date It has not however been the aim of the writer to achieve a stop press effect but rather to present the facts and theories which are at present engaging the attention of those who are especially interested in the function of the endocrine glands

The individual facts and the reasoning of endocrine physiology are not difficult to understand The problem which faces the author is one of presentation to make a lucid statement of that which is known and that which today seems probable At the same time in the course of systematic description it is essential to indicate the limits of present day knowledge with the result that this book has come to contain many references to our incomplete understanding of the subject

Perhaps more than any other aspect of physiology the function of the endocrine glands is indebted to the study of disease However this book is written for those primarily interested in physiology and while the knowledge gained from clinical endocrinology is freely used lengthy descriptions of diseases have been omitted Although the book is intended for graduates as well as undergraduates the language of clinical medicine has been largely suppressed so that those who are unfamiliar with the terminology of disease will not find themselves at a loss

Apart from the knowledge derived from the study of disease endocrine physiology has been constructed on the foundation of experiment Many chapters and papers on the subject are based upon lengthy descriptions of these experiments In the present volume however the conclusions drawn from many such experiments have been incorporated in the text but the current of systematic description has not been interrupted by detailed accounts of experimental procedures In keeping with this policy the references at the end of each chapter will be found to contain only certain selected papers no attempt has been made to support each statement of the text by a series of references Nevertheless at the beginning of most of the lists of references will be found the name of some outstanding monograph or book which gives full references to the literature of the subject concerned

Recent progress in the chemistry of endocrinology cannot be overlooked and to assist in the understanding of this bewildering aspect of the subject, an introductory chapter dealing with elementary organic chemistry has been written This chapter is self contained and the continuity of the book as a whole will not be lost by those who choose to omit it

In recent years the interrelationship between the central nervous system and the endocrine glands has occupied the attention of many workers As a result, a chapter dealing with this aspect of endocrine physiology has been included Much of the work which forms the basis of this chapter is appearing in text book form for the first time perhaps before it has received universal acceptance

Finally I wish to express my gratitude to those authors who have allowed me to reproduce illustrations from their books I am especially indebted to Professor P O Bishop for the help and encouragement he has given me and to Dr R A Melick who has read the book in its various stages and lent invaluable assistance by his stimulating criticism Dr R A Eade of the University of Technology has generously helped with Chapter I and Dr R I Cox with Chapter XIV for which I express my gratitude I also wish to thank Mr Money of Sydney Hospital who has been responsible for photographing many of the illustrations Finally throughout the production of this book, I have received every possible assistance from the publishers

Macquarie Street SYDNEY

Peter F Hall

CONTENTS

INTRODUCTION

The complexity attained by the vertebrate body has brought with it a need for integrating mechanisms responsible for the co-ordinate activity of its several parts This need has been answered by the evolution of two systems—the nervous system and a constellation of endocrine glands The nervous system has made use of filamentous processes by means of which it exerts its influence upon distant parts The endocrine glands on the other hand have elaborated certain chemical structures which affect the tissues of the body to which they are transported in the blood

The essential characteristic of a gland is its capacity to secrete and an endocrine gland is one which delivers its secretion into the blood stream (endon within krino I separate) The active components of such endocrine secretions are called hormones (hormao I arouse)

HORMONES

Although active chemical compounds analogous to the hormones of man exist in the blood of invertebrate animals little is known of their structure and activity Hormones of the type which form the basis of this book are confined to vertebrates

CHEMISTRY The hormones of man are steroid amine protein and amino acid compounds (see Chapter II) The steroids are secreted by cells derived from mesoderm (adrenal cortex testis and ovary) while amine amino acid and protein hormones come from glands which are derived either from ectoderm (adenohypophysis neurohypophysis and adrenal medulla) or from endoderm (thyroid parathyroid and pancreas) The chemical structure of steroid hormones shows little variation between substances which exert widely different effects but the remaining hormones show little in common chemically

SYNTHESIS In the final analysis little is known about the synthesis of hormones in the body Cholesterol appears to be the essential precursor of the steroid hormones and tyrosine is important in the production of thyroid hormone and of adrenaline Too little is known of the structure of the protein hormones to discuss the steps by which they are produced within the body

STORAGE AND RELEASE Hormones are produced within the endocrine cells and are generally released from these cells directly into the circulation However, in the case of the thyroid and to some extent the ovary hormones are stored in special sacs where they await release into the blood stream Some glands, like the thyroid are capable of storing considerable quantities of hormone for long periods while others such as the adrenal gland, store very little hormone It is important to bear in mind that the hormone content of an endocrine gland at a given moment is the outcome of two processes—the rate of secretion and the rate of release of the secretion into circulation This is especially true of those glands which are capable of storing large quantities of hormones If this point be overlooked erroneous conclusions may be drawn from certain experimental data

The activity of glandular structures is frequently reflected by histochemical changes within the constituent cells such as the appearance of intracellular granules In the case of certain endocrine glands (e g the adenohypophysis and the thyroid) these changes constitute valuable circumstantial evidence of activity, whereas in other cases (e g the adrenal cortex) histochemical changes within the cells are more difficult to interpret

TRANSPORT AND INACTIVATION Oestrogens, thyroid hormone and perhaps other hormones are carried in the blood bound to protein Although the significance of this protein binding remains unknown it has been suggested that the attachments of hormones to large protein molecules may decrease the urinary excretion of these compounds by interfering with their passage into the glomerular filtrate In this way protein binding may act as a factor in hormone economy

There is some evidence to suggest that certain hormones do not leave the endocrine cells in their active form but are carried to the tissues upon which they will act where they are chemically altered and thus rendered potent

Still more obscure is the fate of hormones after they have exerted their specific effects It may be that they can be used *over and over again like catalysts Sometimes the parent glands* appear able to inactivate their own hormones while on other occasions the organs upon which a hormone acts are responsible for this inactivation In the case of the steroid sex hormones inactivation takes place in the liver, by the process of conjugation (see page xiii)

A number of hormones when injected into the body over long periods stimulate an antigenic response in the recipient as a

result of which further injections of the hormone in question prove inactive This reaction appears to depend upon the formation of antihormones substances which can in some way antagonise the action of the hormones concerned This form of inactivation applies chiefly to crude preparations of injected protein hormones What part such reactions play under physiological conditions is unknown but there is no reason to believe that antihormones are important in the normal organism

Antihormones are associated with the α globulin fraction of the serum protein and they exhibit the immunological behaviour seen following the injection of protein antigens Antihormones are species specific and minute amounts of these substances have been recovered from certain untreated animals

CONJUGATION Certain tissues (notably the liver) are capable of encouraging the chemical combination between normal body metabolites and certain substances which have resisted the usual metabolic processes of the body This coupling serves as a defence against potentially harmful substances and enables the body to eliminate such undesirable compounds in a physiologically inert state This process of coupling is called conjugation and is illustrated by the union of certain amino acids with toxic substances for example the union of glycine with benzoic acid which gives rise to hippuric acid hippuric acid is relatively non toxic and is excreted in the urine

In addition to the removal of toxic substances the body makes use of the process of conjugation in order to inactivate and eliminate certain substances which are not harmful to the body when present in physiological concentrations For example a number of hormones are excreted in conjugated form and this process prevents the accumulation of an unwanted excess of these substances Conjugated hormones include glucuronides and sulphates Some of these compounds are esters others are ethers

This special meaning of the word conjugation is freely used in the literature of physiology—usually without strict definition Such usage must be clearly distinguished from the use of the word in chemistry where it refers to the disposition of double bonds

EXCRETION Only a small proportion of an injected hormone can be recovered from the urine and only minute amounts of hormones are found in urine under physiological conditions this applies to all hormones To what extent urinary concentrations of hormones and their excretory products reflect the concentrations of these hormones in the blood is unknown For clinical and

some experimental purposes it is convenient to assume that the concentration of certain hormones and their metabolites in urine is a reflection of blood levels but this assumption may not always be justified Some hormones (e g oestrogens) are excreted in small quantities in faeces this excretion is partly biliary and partly intestinal

ACTION OF HORMONES

Hormones activate the rates of specific processes without contributing significant amounts of energy or matter to the tissues involved Their actions fall into three groups—

1 *Morphogenesis* This type of action includes growth metamorphosis and sexual development

2 *Integration* of autonomic activity and instinctual patterns of behaviour This is seen in the control of the sexual and maternal instincts and in the responses of the sympathetic nervous system

3 *Maintenance* of the internal environment This function includes the regulation of the disposition of food stuffs electrolytes and water within the body Included under this heading are the responses of the body to stress (page 44) Hormones appear to be regulatory in their actions and therefore require normal tissues and enzyme systems before they can exert their specific effects

MECHANISM OF ACTION Little is known of the way in which hormones produce their effects They may act as catalysts accelerating reactions which would scarcely proceed in their absence Again it has been suggested that they act by increasing the local concentration of certain enzymes For example parathyroid hormone causes an increase in the alkaline phosphatase content of bone Other examples of local changes in enzyme concentration are known to follow the action of hormones but in some cases the importance of such changes is difficult to assess

SYNERGISM AND ANTAGONISM Sometimes one hormone may assist the action of another while it is antagonistic to that of a third This synergism may not involve every action of a given hormone For example progesterone assists and extends the action of oestradiol upon the breast (synergism), while in their influence upon uterine motility the two hormones are antagonistic Such interrelationships between various hormones may account for certain paradoxical effects observed during experimental studies For example a sudden fall in the concentration of oestrogens in the blood leads to endometrial bleeding This bleeding may be prevented by the concurrent administration of progesterone

but the sudden withdrawal of both hormones at the same time will produce bleeding

The functional state of an endocrine gland may be influenced by that of other glands for example, thyroid hormone stimulates adrenocortical activity In the presence of prolonged overactivity of the thyroid gland this stimulating effect of thyroid hormone upon the adrenal cortex may produce evidence of cortical failure although the adrenal cortex is in fact overacting In other words a disparity may exist between the hormone requirements of the moment and the capacity of a given endocrine gland to meet these requirements

CONTROL OF ENDOCRINE ACTIVITY

Some hormones (e g thyroid hormone) appear to act upon almost every cell of the body Others however select certain tissues for the site of their activities The cells of these tissues are known as the target cells and the tissues as target organs Sometimes the target organ is an endocrine gland and when the principal action of a hormone is to stimulate another endocrine gland it is referred to as a trophic (or tropic) hormone The trophic hormones are secreted by the adenohypophysis it is this capacity to

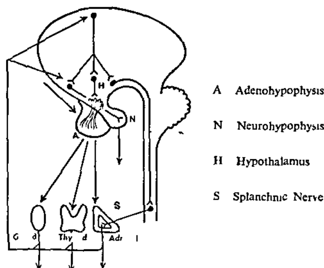


Fig 1 Diagram illustrating the neural control of the endocrine glands The adenohypophysis is shown stimulating the target glands by means of its trophic hormones The effect of the hormones secreted in response to trophic stimulation is exerted upon the nervous system and upon the adenohypophysis The influence of the hypothalamus upon the functions of the adenohypophysis neurohypophysis and adrenal medulla is also illustrated (see Chapter XIII) (*G W Harris*)

secrete such hormones that gives the adenohypophysis the role of director of the endocrine system (Fig 1)

It appears to be the case that the endocrine glands produce a basal level of activity upon which there is superimposed a more rapid rate of secretion when this is required Sometimes this additional activity represents an emergency mechanism, brought into play to restore the internal equilibrium of the body (e g the secretion of adrenaline when the concentration of blood sugar falls below a certain level) Such changes in activity may on the other hand evolve gradually and deliberately as in the secretion of sex hormones during puberty

It will be seen (page 251) that good evidence supports the view that the adenohypophysis is under the control of the nervous system This control appears to be mediated through the hypothalamus In addition however the adenohypophysis is controlled by hormones its trophic secretions stimulate target glands to produce hormones and these hormones themselves depress the secretion of the trophic hormone concerned In this way a check is kept upon the pituitary in such a way that the hormones whose secretion it stimulates, set a limit to the rate of their own production (Fig 1)

So it can be seen that endocrine activity proceeds at various levels The nervous system, receiving impulses from within and without the body controls the activity of the adenohypophysis This gland accordingly stimulates the endocrine structures of the next level by means of its trophic hormones This stimulation calls forth the production of hormones from the target glands which themselves serve to depress the rate of secretion of trophic hormones by the pituitary Finally the hormones produced act upon their target organs and bring about specific changes

The effect of target glands upon the secretion of trophic hormones by the pituitary is reminiscent of the effect of exogenous hormones It is a general rule of endocrine physiology that if a hormone be administered to a normal animal the endogenous production of that same hormone is depressed For example injections of testosterone depress the testicular secretion of this hormone Such interplay between hormones and the glands by which they are secreted makes for a stability and smooth integration of their actions which would not otherwise be possible

METHODS OF STUDY

Although in recent years new techniques have been introduced into experimental physiology which have greatly changed the

methods by which endocrine function is studied much of our knowledge of the glands of internal secretion has been derived from three fundamental lines of investigation

1 *Removal of endocrine glands* The earliest endocrinological experiments consisted of the removal of an endocrine gland and the subsequent observation of the effects produced by this procedure Much has been learnt about the function of the testis the thyroid and the pituitary gland in this way For example the importance of the anterior pituitary gland in growth was first clearly demonstrated by studying the effect of its removal in puppies the hypophysectomised animals failed to reach the adult proportions attained by litter mates

2 *Injection of glandular extracts* The information derived from studies of animals deprived of one or more endocrine glands was strikingly confirmed and greatly elaborated by the use of active extracts of these structures The capacity of testicular extracts to reverse the effects of castration and the restitution of normal health to animals deprived of thyroid tissue following the administration of extracts of this gland are among the early experiments which illustrate the importance of this method of study

3 *Study of disease* Certain diseases may be looked upon as ready made physiological experiments from which much can be learnt about the function of normal glands For example the group of diseases called Cushing s syndrome presents the physiologist with the picture of excessive adrenocortical activity

Since these three methods were first introduced into current physiological practice great refinements have developed Purer preparations of hormones more precise operative techniques and more accurate knowledge of disease have contributed to a better understanding of endocrine function However a great deal more remains to be learnt about the function of endocrine glands under physiological conditions These three methods of study illustrate endocrine activity under abnormal conditions from such experiments only certain aspects of normal function can be understood For example excessive administration of cortisone affects the distribution of adipose tissue throughout the body (page 38) This is seen both during the use of cortisone in the treatment of certain diseases and in the condition called Cushing s syndrome which represents a morbid excess of this and allied steroid hormones However the exact role of cortisone in the normal metabolism of fat is uncertain what effect physiological as opposed to experi

secrete such hormones that gives the adenohypophysis the role of director of the endocrine system (Fig 1)

It appears to be the case that the endocrine glands produce a basal level of activity upon which there is superimposed a more rapid rate of secretion when this is required Sometimes this additional activity represents an emergency mechanism brought into play to restore the internal equilibrium of the body (e g the secretion of adrenaline when the concentration of blood sugar falls below a certain level) Such changes in activity may on the other hand evolve gradually and deliberately, as in the secretion of sex hormones during puberty

It will be seen (page 251) that good evidence supports the view that the adenohypophysis is under the control of the nervous system This control appears to be mediated through the hypothalamus In addition however the adenohypophysis is controlled by hormones its trophic secretions stimulate target glands to produce hormones and these hormones themselves depress the secretion of the trophic hormone concerned In this way a check is kept upon the pituitary in such a way that the hormones whose secretion it stimulates set a limit to the rate of their own production (Fig 1)

So it can be seen that endocrine activity proceeds at various levels The nervous system receiving impulses from within and without the body controls the activity of the adenohypophysis This gland accordingly stimulates the endocrine structures of the next level by means of its trophic hormones This stimulation calls forth the production of hormones from the target glands which themselves serve to depress the rate of secretion of trophic hormones by the pituitary Finally the hormones produced act upon their target organs and bring about specific changes

The effect of target glands upon the secretion of trophic hormones by the pituitary is reminiscent of the effect of exogenous hormones It is a general rule of endocrine physiology that if a hormone be administered to a normal animal the endogenous production of that same hormone is depressed For example injections of testosterone depress the testicular secretion of this hormone Such interplay between hormones and the glands by which they are secreted makes for a stability and smooth integration of their actions which would not otherwise be possible

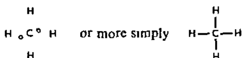
METHODS OF STUDY

Although in recent years new techniques have been introduced into experimental physiology which have greatly changed the

CHAPTER I

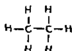
THE CHEMISTRY OF HORMONES

Organic chemistry deals with chemical compounds containing carbon The number of organic compounds already known to exist is so vast that chemists have developed an elaborate system of nomenclature which enables the structure of a compound to be indicated by its name Some of these names however are cumbersome so that many compounds also bear simpler names which are called trivial names In the case of hormones trivial names are much more familiar than their systematic counterparts In addition to this system of nomenclature there has been evolved a series of symbols to indicate the molecular structure of organic compounds The carbon atom has four valencies and the simplest series of organic compounds are the hydrocarbons which contain only carbon and hydrogen The first member of this series is methane —



Methane CH_4

The four available electrons of carbon are associated with the single available electron of each of four hydrogen atoms The next compound of the same series is ethane —



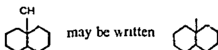
II Ethane C_2H_6

Methane and ethane are fully saturated because all four valencies of the carbon atoms have been used up the suffix ane indicates saturation When a molecule is unsaturated unused valencies

mental concentrations of the hormone exert upon fat metabolism cannot be determined from such observations alone

Because of the great advances made in the chemical methods which form the basis of present day knowledge of hormones it has become desirable to discuss the function of endocrine glands *in terms of the chemistry and action of the hormones which they* secrete This approach is essentially different from that previously adopted which consisted to a large extent of descriptions of the three classical methods of investigation—the effect of removing a gland the effect of glandular extracts and the study of disease

In the case of steroid nuclei a methyl group attached to the C atom at the junction of two rings may be indicated simply by a stroke e g —



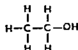
These abbreviations make structural formulae much simpler and enable minor differences in the functional groups of a molecule to be indicated more clearly An organic compound may be considered to lose one hydrogen atom and the molecule after this hypothetical loss is referred to as a radical or group Such a group is named by means of the suffix yl e g —



Such radicals are free to combine with other organic compounds Certain groups of atoms may replace one or more of the hydrogen atoms in a hydrocarbon molecule and the resulting compounds will possess different physical and chemical properties from those of the parent molecule Some of the important types of compounds resulting from such substitution are —

1) Alcohols

Alcohols contain the group OH and are named either by the suffix -ol or the prefix hydroxy-

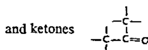


X Ethanol or hydroxyethane

2) Aldehydes and Ketones

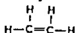
Aldehydes and ketones contain the carbonyl group (—C=O)

Aldehydes (except formaldehyde) have the structure —C—C(H)=O



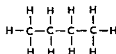
The oxygen uses up two of the valencies of carbon while the two remaining valencies are used up by two other atoms one of which is always hydrogen in the case of aldehydes both of which are carbon in the case of ketones e g —

are shared and this is indicated by a double bond (C = C) e g —

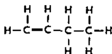


III Ethene C_2H_4

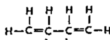
The suffix -ene refers to the unsaturated nature of a compound diene and triene indicate the presence of two and three double bonds respectively The position of a double bond is indicated by the lower number of the two carbon atoms so joined, two double bonds are indicated by the two numbers, with the smaller first e g —



IV Butane



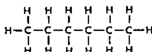
V 1 Butene



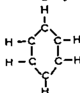
VI 1 3 Butadiene

Unsaturated compounds are usually very reactive because of the readiness with which the unused valencies can be taken up by the introduction of other atoms or groups into the molecule

Such hydrocarbons as those mentioned above are said to possess an open chain structure the carbon atoms being linked together in a straight chain Hydrocarbons can occur however with their carbon atoms in cyclic or ring form e g cyclohexane —

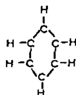
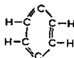


VII Hexane



VIII Cyclohexane

Five membered and six membered rings are common examples of such cyclic compounds In writing the formulae of ring compounds it is convenient to omit the C atom at each angle of the ring and hydrogen atoms attached to these carbon atoms are not usually indicated unless they are especially important to the structure of the molecule e g —

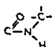
 *may be written*  *and*  as 

VIII Cyclohexane IX Benzene

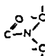
the product is called an amide Like amines, amides are of three types—primary secondary and tertiary —



Primary amide

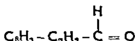


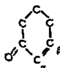
Secondary amide



Tertiary amide

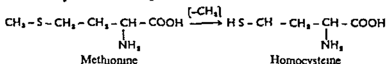
In the case of organic compounds whose molecules contain functional groups the carbon atom next to that which bears the active group is referred to as the α carbon atom and that next to an α carbon atom as the β carbon atom, e g —





In the second example the functional group is a ketone and the α and β carbon atoms are joined by a double bond This state of affairs gives rise to important functional changes in the molecule and is referred to as $\alpha\beta$ unsaturation

TRANSMETHYLATION The reaction of transmethylation involves the donation of a methyl group (CH_3-) from the molecules of one compound to those of another The amino acid methionine is the chief donor in such transmethylations being transformed into homocysteine in the process —

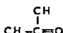


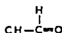
Methionine Homocysteine

Transmethylation enables the body to add a CH_3- group to a compound without the need for synthesising such a group The methylation of nor adrenaline which gives rise to adrenaline is an example of this reaction (page 182)

CHEMISTRY OF HORMONES

The known hormones are either steroids proteins amino acids peptides or amines The steroids include all the known hormones of the adrenal cortex and of the gonads The protein hormones include all the hormones of the adenohypophysis the hormone of the parathyroid glands and insulin The hormones of the neurohypophysis are polypeptides while those of the adrenal





XI Acetone or propanone XII Acetaldehyde or ethanal

Ketones are named by applying the suffix -one aldehydes by -al The presence of a hydrogen atom attached to the C = O group of aldehydes and its absence in the case of ketones accounts for some of the differences between these two groups of compound

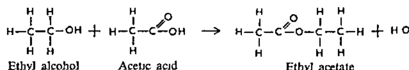
3) Acids

Organic acids contain the carboxyl group $-C(=O)-OH$ or as it is often written COOH e g — $CH-C(=O)-OH$ $CH-CH-C(=O)-OH$

XIII Acetic acid XIV Propionic acid

4) Esters

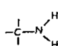
The reaction of an alcohol with an organic acid to give an ester and water is reminiscent of the inorganic reaction of alkali and acid to give a salt and water Esters are named in much the same way as inorganic salts, e g —

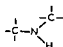


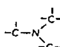
In certain hormones the reaction between an OH group and an organic acid may produce an ester which shows certain desir able properties e g testosterone propionate

5) Amines

Amines may be regarded as substituted ammonias They are divisible into primary secondary and tertiary amines according to the number of carbon atoms attached directly to the nitrogen atom of the amine group —



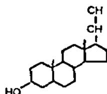




Primary amine Secondary amine Tertiary amine

6) Amides

When the OH of the acid radical $-C(=O)-OH$ is replaced by NH

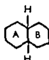


XX Pregnan 3-ol

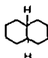
If more than one such substituent is present it is usual to designate that attached to the lowest carbon atom number first e g pregnan 3 ol 16 17 dione The final e of the parent compound is dropped if the adjacent suffix begins with a vowel i e pregnan 3-ol pregnane 3α 17α 20α triol

Double bonds The existence of a double bond is indicated by the symbol Δ and its position by a superscript which denotes the lower number of the two carbon atoms so joined e g Δ^4 means a double bond between C_4 and C_5 If a single number be ambiguous (e g Δ^8 could mean $C_8 = C_9$ or $C_8 = C_{14}$) both numbers are given thus — $\Delta^{8\,9}$ The presence of more than one double bond as opposed to the complete designation of one double bond will be made evident by the accompanying suffix -ene diene triene etc

Isomerism of carbon atoms common to 2 rings When saturated rings are fused (i e have two C atoms in common) two isomers are possible depending upon whether the two hydrogen atoms attached at the junctions of the rings are on the same or opposite sides of the plane of the rings concerned e g —



or



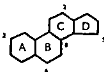
Both H atoms above the plane of the paper

One H atom above and one below the plane of the paper

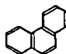
This type of isomerism among naturally occurring steroids occurs only at the junction of rings A and B and hence only carbon atoms 5 and 10 are involved When the two substituents are on the same side of the plane of the nucleus (cis) the compound is named without any additional prefix (e g pregnane) When the two substituents are on opposite sides (trans) the prefix allo is used e g allopregnane

medulla are amines Thyroid function is concerned with certain amino acids peptides and proteins

STEROIDS A steroid may be defined as an organic compound which yields methylcyclopentanophenanthrene on dehydrogenation with selenium This is a more precise way of saying that steroids possess a common ring system —



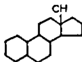
XV Fundamental ring system of steroids



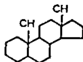
XVI Phenanthrene

This is called a perhydrocyclopentanophenanthrene nucleus and consists of 3 six membered rings as in phenanthrene to which is attached a five membered ring (cyclopentane) The carbon atoms are numbered in the way illustrated in XV and the rings are referred to as A B C and D

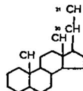
The steroid hormones include the sex hormones and the hormones of the adrenal cortex They are divisible into three groups each derived from a parent hydrocarbon structure—oestrane androstane and pregnane —



XVII Oestrane



XVIII Androstane



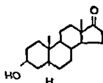
XIX Pregnane

The carbon atoms of side chains are numbered as shown in the case of pregnane (XIX)

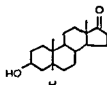
Nomenclature of steroid hormones When a hydrogen atom attached to a carbon atom is substituted by another atom or group of atoms the number of that carbon atom is placed before the prefix or suffix which designates the nature of the substituent e g pregnan 3 ol means that a hydrogen atom attached to C_3 of pregnane has been replaced by an OH group Some authors place the number after the substituent —pregnanol 3

adjacent to a functional group Whether these symbols indicate a structural configuration or the carbon atoms adjacent to a reactive group, will be clear from the context in which they are used

Epi The prefix epi- refers to isomers which differ only in the stereic arrangement of the groups about one carbon atom Some workers use the prefix iso- instead of epi but the latter is preferred because it is more specific e g androsterone and epiandrosterone —



XXV Androsterone



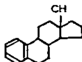
XXVI Epiandrosterone

SEX HORMONES

OESTROGENS The natural oestrogens are derivatives of oestrane Three are known (oestrone oestradiol and oestriol) and all three are unsaturated compounds The unsaturation involves three double bonds in the A ring which thereupon becomes a benzene type (benzenoid) ring —

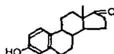


To accommodate this unsaturation the methyl group at C_{10} must disappear and so the natural oestrogens are derived from oestratriene —

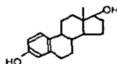


XXVII Oestratriene

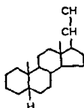
They are as follows —



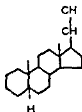
XXVIII Oestrone or oestratrien 3-ol 17-one



XXIX Oestradiol or oestratriene 3 17-diol



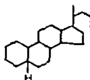
XXI Pregnane



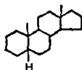
XXII Allopregnane

The methyl groups attached to C_{10} and C_{13} are on the same side of the nuclear plane in the case of all naturally occurring steroids and they are simply designated by solid lines (CH_3 being omitted) because it is convenient to assume that they lie above the nuclear plane rather than below

Etio The prefix etio- refers to the simplest compound that can be obtained by degradation of a parent substance which still retains the ring system and fundamental chemical characteristics of the parent substance e g —



XXIII Cholane

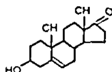


XXIV Etiocholane

α and β configurations It is customary to regard the two methyl groups attached to C_{10} and C_{13} as being in a plane above that of the nucleus i e in the structural formula these groups are considered to lie above the plane of the paper Substituents at C_3 C_{11} or C_{17} may be on the same or opposite side of the nucleus in relation to the C_{10} and C_{13} methyl groups Substituents on the same side as these two methyl groups are said to possess a β configuration which is indicated by a solid line joining the substituent to the nucleus substituents on the opposite side are attached by a broken line and possess an α configuration In naming such compounds α or β is written in brackets after the carbon atom involved The symbols α and β are more precise than the terms cis for (β) and trans for (α) because these prefixes are used in other contexts

It should be noticed that this use of the symbols α and β to indicate the structural configuration of organic compounds is quite distinct from the use of the same symbols encountered on page 5 where they are used to designate the carbon atoms

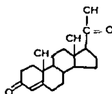
Dehydrogenation of epiandrosterone at C_5 and C_6 produces dehydroepiandrosterone —



XXXIV Dehydroepiandrosterone i e Δ^5 androsten 3(β)-ol 17-one

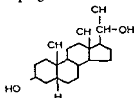
17 Ketosteroids Among the end products of the metabolism of adrenal and testicular androgens are a number of steroid com pounds which bear a ketone group at C_{17} (called 17 ketosteroids) One way in which the adrenal and testicular components of the urinary 17 ketosteroids may be differentiated depends upon the fact that the OH group attached to C_3 may exhibit either an α or a β configuration and so there are two fractions (α and β) of urinary 17 ketosteroids and these can be distinguished by chemical means It is believed that the β fraction is chiefly derived from adrenal androgens Dehydroepiandrosterone is the most important constituent of the β fraction

PROGESTERONE Progesterone and its metabolites are named as derivatives of pregnane Progesterone is the trivial name for Δ pregnene 3 20 dione —



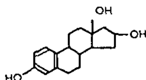
XXXV Progesterone

Among the metabolites of progesterone which appear in the urine is pregnanediol or pregnane 3α 20α diol —



XXXVI Pregnanediol

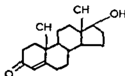
Another is pregnan 3α ol 20 one These two metabolites together constitute the pregnanediol complex (page 115)



XXX Oestriol or oestratriene 3 16(β) 17(α) triol

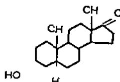
It will be seen that the OH group attached to C_3 in these three molecules can occur only in the same plane as that occupied by the A ring (because of the double bonds in that ring) and hence α and β isomers do not exist

ANDROGENS Androgens may be named as derivatives of androstane Testosterone is the trivial name of the testicular hormone —



XXXI Testosterone

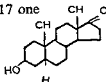
The systematic name of this compound is Δ^4 androsten 17β ol 3 one
Methyl testosterone is active when taken by mouth and has a methyl group attached to C_1 The most important metabolite of testosterone is androsterone —



XXXII Andro tan 3(α)-ol 17-one (Androsterone)

Another important androgen is epiandrosterone which differs from androsterone only in that the OH group at C_3 occupies the β position

i e androstan-3(β) ol 17 one



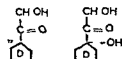
XXXIII Epiandrosterone

All active adrenocortical steroids (except the sex hormones) have two structural features in common —

1)

(A) C_3 bears a ketone group and a double bond exists between the α and β carbon atoms (α β unsaturated ketone)

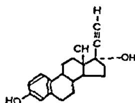
2)



The side chain attached to C_{17} contains an alcohol and a ketone grouping This side chain like the OH at C_{11} always occupies the (β) position It therefore follows that in 17-hydroxy compounds the 17 OH occupies the (α) position since two substituents attached to one carbon atom cannot possess the same spatial configuration

DERIVATIVES OF STEROID HORMONES Some derivatives of steroid hormones are widely used in clinical practice —

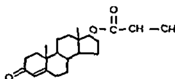
Ethinyl oestradiol The ethinyl radical may be represented thus — H – C ≡ C –



XXXVIII Ethinyl oestradiol

This steroid is a potent oestrogen of low toxicity

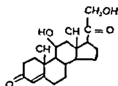
Testosterone Propionate If the OH at C_{17} of testosterone combines with propionic acid (CH_3 CH COOH), testosterone propionate results —



XXXIX Testosterone propionate

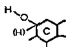
ADRENOCORTICAL STEROIDS

The active adrenocortical steroids, other than the sex hormones should logically be named as derivatives of pregnane However, corticosterone is the trivial name given to one such steroid and the remainder are named as derivatives of this compound —

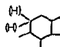


XXXVII Corticosterone

Deoxy (desoxy) compounds are those which possess one oxygen atom less than the reference substance (i e the compound from whose structure a group of substances is named) Thus deoxycorticosterone differs from corticosterone by the absence of the oxygen atom at C_{11} —



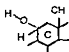
Corticosterone



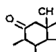
Deoxycorticosterone

The hydrogen atoms shown in brackets would not normally appear in the usual structural formulae

Dehydro compounds are those which possess two hydrogen atoms less than the reference substance Usually this involves the loss of two hydrogen atoms from an alcohol to give the corresponding ketone e g dehydrocorticosterone differs in this way from corticosterone —



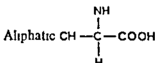
Corticosterone

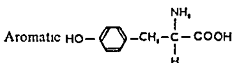


Dehydrocorticosterone

Dehydrogenation may however involve the loss of one hydrogen atom from each of two adjacent carbon atoms to give a double bond e g epiandrosterone and dehydroepiandrosterone (see page 11)

possess more carboxyl than amino groups and basic amino acids possess more amino than carboxyl groups In each of these types the amino acids may be aliphatic, where R is an open chain component or aromatic when R possesses a benzene ring e g —



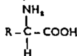


XLI Alanine or α aminopropionic acid

XLII Tyrosine or α-amino-β [p-hydroxyphenyl] propionic acid

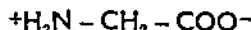
Most amino acids are known by trivial names (alanine tyrosine etc) but they also bear systematic names in which the term α amino precedes the name of the acid from which they are derived (e g in XLI and XLII above CH_3 CH_2 COOH propionic acid)

Optical Activity of Amino Acids In the general formula



it will be seen that except in the case of glycine (where R is hydrogen) the α-carbon atom will bear four substituents each of which is different from the other three In this way the α-carbon atom is a centre of asymmetry and so all naturally occurring amino acids (except glycine) are optically active that is they cause rotation of the plane of plane polarised light when this is passed through solutions of these amino acids The direction of rotation is indicated by (*d*) or (+) on the one hand and (*l*) or (-) on the other These symbols should be distinguished from the letters D and L which are used to indicate the configurational relationships existing between similar compounds e g L alanine is dextrarotatory but possesses the same configuration as L glyceraldehyde

Amino Acids as Electrolytes Since all amino acids contain at least one carboxyl and one amino group they behave as weak acids and as weak bases A simple molecule such as glycine is ionized in such a way that it possesses an equal number of positive and negative ions —



$$^{+}H_3N - CH_2 - COO^{-}$$

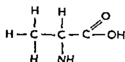
In this condition the molecule can be regarded as electrically neutral The pH at which such a dipolar ion does not migrate in an electric field is called the isoelectric point At this pH if solutes

TABLE I

Meaning of suffixes and prefixes used in naming organic compounds

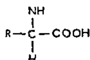
Suffix	Meaning
ane	Saturated hydrocarbon
ene	Unsaturated hydrocarbon i e possessing double bonds
ol	*Alcohol or OH group*
one	Ketone or C = O group
Prefix	*Meaning*
hydroxy	*OH group (alternative to ol)*
oxo	*Ketone or C = O group*
allo	Other—refers to one of two isomers
cis	The arrangement of two groups on the same side of a *molecule*
trans	The arrangement of two groups on opposite sides of a molecule
etio	Refers to the final degradation product of a more complex molecule which still retains the essential character of the original molecule
iso	Refers to one of two isomers
epi	Refers to one of two isomers which differ from each other only in the spatial arrangements of groups about one carbon atom
deoxy	An oxygen atom lost
dehydro-	Two hydrogen atoms lost

AMINO ACIDS The important amino acids of the body consist of organic acids in which a hydrogen atom attached to the carbon atom next to that which bears the acid group (α carbon atom) is replaced by an *amine group* (NH_2) *e g* —



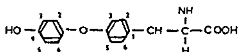
XL Alanine or α aminopropionic acid

α amino acids may be represented by the general formula

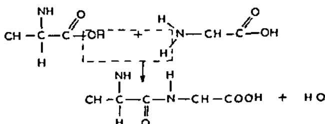


where R is the component which distinguishes one such amino acid from another The usual classification of amino acids depends upon the relative number of acidic and basic groups present in the molecule Neutral amino acids contain an equal number of amino (NH_2) and carboxyl (COOH) groups acidic amino acids

The system of numbering the carbon atoms in the thyroxine nu-leus should be noticed —



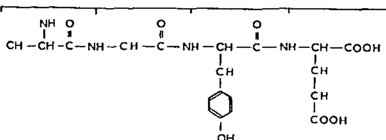
PEPTIDES Peptides may be defined as polyamides of low mole cular weight which yield two or more amino acids on hydrolysis The constituent amino acids are linked by a reaction between the NH_2 and COOH groups of adjacent amino acids —



This —C—N linkage is called the peptide or amide linkage

Peptides are built up through a series of such linkages and the terms di tri and tetrapeptides indicate the number of amino acids which go to make up a given peptide molecule it is usual to refer to peptides containing three or more amino acids as polypeptides

Peptides are named by listing the constituent amino acids each amino acid taking the suffix yl except that which bears the unsubstituted acid e g —

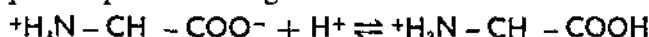


Alanylglycyltyrosylglutamic acid

It is common practice to indicate the nature of a polypeptide by

other than water and other ions be absent the number of cations will equal the number of anions the pH at which this state of affairs exists is called the isoionic point Variations in the solute and the presence of other ions will cause the value of the isoelectric point and that of the isoionic point to differ one from the other

If hydrogen ions be added to a solution of glycine the ionization of the acid radical will be suppressed and the molecule as a whole will acquire a positive charge —

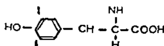


$$^{+}H_3N-CH_2-COO^{-} + H^{+} \rightleftharpoons {}^{+}H_3N-CH_2-COOH$$

The opposite state of affairs will result from addition of base In this way it becomes evident that an amino acid at the isoelectric point is to be regarded as a salt which is fully ionized and internally neutralised by its own acidic and basic groups The isoelectric point is an important property of amino acids and of proteins since many physical and chemical properties of these substances change as the pH moves one way or the other from that of the isoelectric point

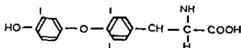
The following amino acids are important in the secretions of endocrine glands —

(I) Iodotyrosines e g —



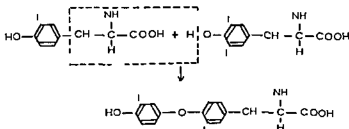
XLIII 3 5-diiodotyrosine

(II) Iodothyronines, e g —



XLIV 3 5 3 triiodothyronine

In Chapter III it will be shown that triiodothyronine may be formed from the union of one molecule of monoiodotyrosine with one molecule of diiodotyrosine This union involves the loss of one side chain —



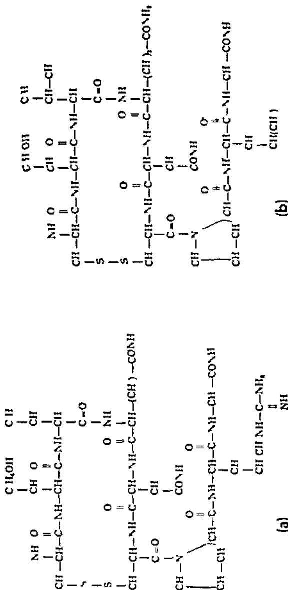


Fig 2 The chemical structure of (*a*) antidiuretic hormone and (*b*) oxytocin

simply writing the sequence of its constituent amino acids e g —

x ⌐alanine glycine tyrosine glutamic acid, or simply — Al Gly Tyr Glu x being used to denote the site of the free amino group Such systematic names are so cumbersome that most polypeptides of biological importance are commonly known by trivial names, e g oxytocin vasopressin etc

The important polypeptide hormones of the body are oxytocin and vasopressin (ADH), each of which contains eight amino acids of which six are common to both (Fig 2) Each hormone yields three molecules of ammonia on hydrolysis because aspartic and glutamic acids occur in these molecules in the form of their amides asparagine and glutamine respectively while the terminal glycine of each peptide is present as glycinamide i e these hormones are octapeptide amides Oxytocin has the following structure —

L-cysteinyl L tyrosyl L isoleucyl L glutaminyl L asparaginyl L-cysteinyl L-prolyl L leucylglycinamide

Vasopressin can be represented as follows —

L cysteinyl L-tyrosyl L phenylalanyl L glutaminyl L asparaginyl -L-cysteinyl L prolyl L-arginylglycinamide

PROTEINS Proteins are complex organic compounds which yield a amino acids on hydrolysis Proteins consist of larger and more complex molecules than peptides, but both consist essentially of amino acids which have been joined by peptide linkages Synthesis of proteins is an undertaking at present beyond the resources of chemists and protein chemistry consists largely in the examination of the individual parts of the molecule their arrangement and the chemical and physical properties of the whole molecule Therefore instead of meeting problems of nomenclature and considerations of structural formulae such as those encountered in steroid chemistry we are here concerned with physical and chemical properties of the protein molecules and something of the arrangement of their component parts

In general proteins contain 45 to 55 per cent carbon 6 to 8 per cent hydrogen 19 to 25 per cent oxygen and 14 to 20 per cent nitrogen They consist of very large molecules with molecular weights from about 10 000 to many millions Like their constituent amino acids they are dipolar ions capable of combining with both acids and bases In addition proteins are very labile and are greatly altered by changes in pH by heat and by the presence of organic solvents

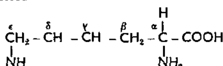
It is believed that peptide chains are folded into precise patterns which are maintained by such hydrogen bonds as those shown in XLV These considerations suggest that the shape of protein molecules is highly characteristic and significant in the role they play in animal physiology

The protein hormones of the body include the hormones of the adenohypophysis of the islets of Langerhans and of the para thyroid glands

ADENOHYPOPHYSIS

GROWTH HORMONE Growth hormone is a protein and has been obtained in crystalline form It has a molecular weight of 49 000 and an isoelectric point at pH 6 85 This protein appears *to be homogeneous when subjected to electrophoretic and other* studies It is relatively stable in alkaline media but is rapidly inactivated by heat acid or proteolytic enzymes

The biological activity of growth hormone appears to depend upon the integrity of certain ε amino groups such as that of lysine but not upon that of the α amino groups of this and other amino acids in the molecule



Lysine (a diamino acid)

The tendency shown by growth hormone to become associated with protein mixtures and to be removed from these only with difficulty is reminiscent of the behaviour of haemin when added to such mixtures but there is no evidence to suggest any similarity in the structure of these two compounds[1]

THYROID STIMULATING HORMONE Chemically pure thyroid stimulating hormone has not so far been prepared A purified thyrotrophic fraction from beef pituitary has been studied *and found to show the properties* of a protein This substance has a molecular weight of approximately 10 000 and gives a positive Molisch reaction indicating the presence of carbohydrate within the molecule The protein nature of the substance is confirmed by the destructive action of proteolytic enzymes in vitro and there is further histochemical evidence that it is a glycoprotein (see page 140)

ACTH ACTH which has been isolated in a high degree of purity from several species is a protein of molecular weight 20 000

Finally, proteins are very reactive and specific in their behaviour owing chiefly to the active groups and side chains of the component amino acids

Classification Proteins are classified as (i) simple proteins which yield only amino acids on hydrolysis and (ii) conjugated proteins which yield in addition other groups called prosthetic groups Examples of conjugated proteins are nucleoproteins which on hydrolysis give nucleic acid, glycoproteins which give carbohydrate and phosphoproteins which give phosphoric acid

Properties 1 Like amino acids proteins are amphoteric and migrate in an electrical field The direction of migration is determined by the nett charge of the molecule A protein molecule does not migrate at its isoelectric point

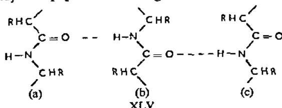
2 As acids or bases proteins can bind ions protein anions can bind with cations protein cations with anions

3 The electrophoretic behaviour of a given protein is characteristic and is useful in separating mixtures of proteins

4 The solubility of proteins is highly characteristic and is influenced among other factors by pH In general, solubility is at a minimum at the isoelectric point The solubility of a protein is an extremely sensitive index of its purity

5 The determination of the molecular weight of a protein can be achieved by a number of methods and the result is an important characteristic in the identification of a given protein A number of proteins have been shown to consist of mixtures of molecules having different molecular weights

6 Shape of protein molecules Most proteins contain more than one peptide chain and therefore cross linkages involving bonds other than peptide links must occur It is known that the S S bond of cysteine is one such method of bonding Another type of bond is that resulting from the attraction between peptide bonds of adjacent peptide chains e g —



XLV

where (a) (b) and (c) represent parts of three adjacent peptide chains

glycoprotein, but the chemistry of the LH isolated from swine pituitary and that from the sheep differ to a significant degree The protein isolated from sheep hypophyses has a molecular weight of 40 000 and an isoelectric point at pH 4 6

PROLACTIN Prolactin was the first of the adenohypophysial hormones to be isolated in a highly purified state It has been obtained in crystalline form and has the properties characteristic of a protein with a molecular weight of 32 000 and an isoelectric point at pH 5 7 The molecule does not appear to contain carbohydrate and partial hydrolysis has not succeeded in producing biologically active peptide fractions which suggests that its endocrine activity is dependent upon the protein molecule as a whole

PANCREAS

INSULIN Insulin can be obtained in highly purified crystalline form and has been more fully studied chemically than other protein hormones As a result of these studies the enormous task of identifying the sequence of amino acids which go to make up the two component peptide chains of the molecule (referred to as Chain A containing 21 amino acids and Chain B containing 30 amino acids) has been achieved

Insulin has a molecular weight of 12 000 and is isoelectric at pH 5 4 The hormone is an example of a protein in which acidic groups predominate over basic groups with the result that insulin readily combines with certain basic proteins such as protamines and globin The resulting products have certain useful properties which have been exploited in using the hormone in the treatment of the disease diabetes mellitus

PARATHYROID GLANDS

PARATHYROID HORMONE The results of attempts to purify parathyroid hormone have so far been unsatisfactory However it would appear that the active component is a protein or is very closely associated with a protein The extraction and purification of the hormone as far as they have gone involve methods appropriate for proteins The best preparations are heterogeneous and show an isoelectric point at pH 5 0 to 6 0 and a molecular weight of the order of 650 000 Biological activity disappears when these preparations are subjected to acid or alkaline hydrolysis or to the action of proteolytic enzymes

REFERENCE

1 Reid E J Endocrinol 8 50 1952

and isoelectric point at pH 4 6 At first ACTH appeared to be homogeneous when subjected to the usual physico-chemical examinations (electrophoresis, ultracentrifugal and solubility behaviour) More sensitive measures (countercurrent distribution and chromatography), together with studies of partial hydrolysis indicate that the original ACTH protein consists of several dissociable components which are peptides of molecular weights from 1 200 to 5 000 some of these peptides are capable of stimulating the adrenal cortex

These and other investigations have suggested that the protein fraction originally isolated is a carrier of the active principle In fact it has been suggested that there exist two corticotrophins one capable of stimulating adrenal weight (AWF) and the other of depleting the ascorbic acid content of the cortex (AAF) The action of AWF may be part of the action of growth hormone or of some factor closely associated with this hormone It is impossible at present to give a final answer to the exact nature of corticotrophin but the efforts to isolate purer compounds have resulted in three principal fractions—

1 A larger protein molecule called crude corticotrophin This is the preparation used clinically and called U S P ACTH

2 Corticotrophin A prepared from glacial acetic acid extracts of pituitary tissue

3 Corticotrophin B prepared by peptic hydrolysis of crude corticotrophin Corticotrophin A and B are straight chain polypeptides each consisting of 39 amino acids

FOLLICLE STIMULATING HORMONE Follicle stimulating hormone has been isolated in highly purified form The substance is a protein of molecular weight of approximately 6 700 and an isoelectric point at pH 4 5 On hydrolysis FSH yields not only amino acids, but some carbohydrate (hexose and hexosamine), indicating that it is a glycoprotein a fact confirmed by its staining reactions (see page 140)

It is interesting to notice that while trypsin hydrolysis destroys the biological activity of FSH pepsin leaves a potent follicle stimulating residue in the dialysable fraction of the hydrolysate This observation has prompted attempts to prepare smaller peptide fractions of such hormones as FSH which might exhibit biological activity since the task of synthesising the whole protein molecule is beyond present resources

LUTEINIZING HORMONE Luteinizing hormone has also been isolated in a high degree of purity It shows the properties of a

The adrenal cortex is essential to life and when all cortical tissue is removed from an experimental animal it enters a physical decline a few days after operation and dies within two weeks Among the changes seen during this period anorexia vomiting diarrhoea loss of weight and a fall in blood pressure are prominent At the same time such adrenalectomised animals are extremely susceptible to noxious stimuli (heat cold trauma injection etc) Certain biochemical changes are also evident before death Serum sodium and chloride levels fall and serum potassium rises Haemo-concentration and evidence of renal failure also appear while excess of water proves toxic to the adrenalectomised animal These changes are alleviated by feeding the animal with large doses of salt and water

Embryology

The cortex in common with the gonads is derived from mesoderm situated between the urogenital fold and the dorsal mesentery In the human embryo of 5 weeks proliferation of cells near the root of the mesentery is evident and eventually prominent vascular masses project between the mesonephros and the mesentery (Fig 3)

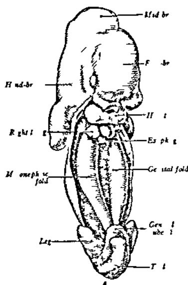


Fig 3 Urogenital folds of the human embryo of six weeks seen from the ventral surface after removing the overlying body wall and viscera (Arey *Developmental Anatomy* Saunders)

CHAPTER II

THE ADRENAL CORTEX

Introduction

The adrenal or suprarenal glands are composite structures in mammals and represent the fusion of two separate glands—the adrenal medulla and the *adrenal cortex*. In elasmobranch fish these two structures are quite separate while in reptiles the two components are indiscriminately mixed—islets of cortex and medulla being scattered throughout the gland. The close anatomical relationship between these two structures appears to be so deliberate as to suggest some functional unity. It may be that the work of Selye dealing with the reactions of the body to conditions of stress may provide some foundation for this suggestion. Stress elicits a response from both the cortex and the medulla, a fact which may have some bearing upon their proximity one to the other. Apart from the role played by the adrenal cortex in the responses of the body to conditions of stress it is also concerned with the metabolism of minerals, water and glucose. In addition the gland produces sex hormones.

In recent years a great deal has been learnt about the function of the cortex from perfusion studies. Such experiments are performed upon the isolated gland. The perfusing fluid is fed into the arterial supply of the gland and changes in its composition are studied in samples taken from the adrenal vein. The importance of this method lies in the fact that the cortex stores very little hormone, producing its secretions to meet the needs of the moment. It is for this reason that extracts of cortical tissue contain a number of hormone precursors and the distinction between an active hormone and a chemically similar precursor is only possible when the chemistry of cortical extracts is interpreted in the light of studies of adrenal vein blood. At least 29 distinct chemical substances can be isolated from the cortex but only a few of these appear in the blood of the adrenal vein. The remaining substances are thought to be precursors formed during the synthesis of the final hormones although in some instances they are physiologically active.

anywhere within the abdomen Such adrenal rests are sometimes important when the adrenal glands are the site of disease and tumours occurring in ectopic cortical tissue are not unknown

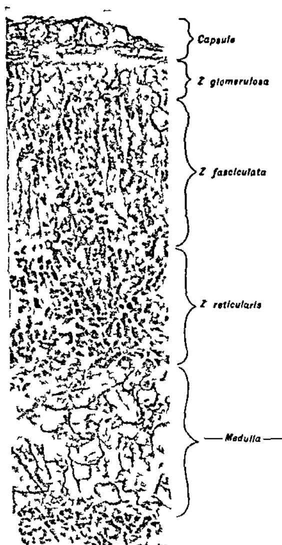


Fig 4 The histology of the adrenal cortex (*Maximow and Bloom*)

During the third month of intrauterine life the cortex reaches its greatest relative size exceeding that of the kidney At this stage the bulk of the cortex consists of sheets of large eosinophil cells which do not show the staining reactions of lipoids this mass of cells is called the X zone

At birth a thin layer of lipoid staining cells is seen where the zona glomerulosa will eventually develop and the whole gland is about one quarter of the weight of the kidney In adult life, one adrenal is about one thirtieth of the weight of one kidney

Beginning shortly before birth the X zone undergoes an extra ordinary process of degeneration which causes the foetal cortex to shrink The debris resulting from this process is replaced by loose connective tissue while at the same time a layer of lipoid staining cells becomes more prominent This process of degenera tion becomes more intense just after birth the significance of these changes is not understood

Histology

The cortex is deep yellow in colour and occupies three-quarters of the width of the whole adrenal gland on cross section The component cells of the cortex are arranged in three layers named from without in —Zona glomerulosa Zona fasciculata and Zona reticularis (Fig 4)

The zona glomerulosa *is the narrowest of these zones and its cells appear in ill defined clusters The zona fasciculata is the* broadest of the three and *its cells are arranged in parallel bands* The *zona reticularis is probably derived from the X zone of the foetal cortex its cells possess fine lipoid droplets together with* pigment granules

Accessory Adrenal Tissue

The occurrence of accessory cortical and medullary tissue together or the occurrence of one or both adrenal glands in an ectopic position although not uncommon in certain animals is of great rarity in man On the other hand accessory cortical tissue by itself is not so rare and in small amounts may be found in as many as 50 per cent of newborn human infants Usually this acces sory tissue undergoes atrophy but in some cases it may persist Typical sites in which such accessory tissue may be found include the perirenal fat the kidney, the liver and along the path of descent of the gonads However cortical tissue may occur almost

Chemistry of Adrenocortical Hormones

1 *SEX HORMONES*

(a) *Androgens* Adrenocortical extracts contain at least six androgens which of these are secreted into the adrenal vein is uncertain Five of these androgens belong to the series of nineteen carbon atom steroids while the sixth is a derivative of pregnane and therefore has 21 carbon atoms The chemical names of these compounds are as follows—

(i) Δ^4 Androstene 3 11 17 trione (called adrenosterone)

(ii) Δ^5 Androsten 3β ol 17 one (called dehydroepiandrosterone or dehydroisoandrosterone)

(iii) Δ^4 Androstene 3 17 dione (called androstenedione)

(iv) Androstane 3β 11β diol 17 one

(v) Δ^4 Androstene 11β ol 3 17 dione

(vi) 17α hydroxyprogesterone

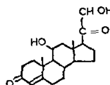
It seems likely that 17α hydroxyprogesterone is a precursor of corticosteroid hormones while the C compounds represent the important cortical androgens 17α hydroxyprogesterone was originally called 17β hydroxyprogesterone but most workers now agree that the compound possesses the α configuration

(b) *Oestrogens* Oestrone has been identified in extracts of cortical tissue but its significance remains to be determined

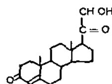
(c) *Progesterone* itself is found in the adrenal cortex and is an important intermediary in the synthesis of corticosteroids

2 *CORTICOSTEROIDS* Seven of the steroids so far isolated from cortical extracts are known to exert important effects upon the metabolism of glucose minerals and water

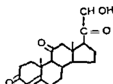
These hormones are —



Corticosterone (Compound B)



11-deoxycorticosterone



11-dehydrocorticosterone (Compound A)

Function

The function of the adrenal cortex is to secrete the following hormones

1 Sex hormones (a) Androgens
(b) Oestrogens
(c) Progesterone

2 Corticosteroids (a) Glucocorticoids
(b) Mineralocorticoids

These hormones are all steroids and so far as is known the adrenal cortex has no function other than the secretion of these steroids More than 29 crystalline steroids have been isolated from adrenocortical tissue extracts In addition to these hormones extracts of adrenocortical tissue contain two groups of compounds, one of which is called the amorphous fraction because it contains physiologically active substances of unknown composition The second group of compounds is called the inert fraction It is thought that these two fractions consist of precursors or degradation products of active hormones The term glucocorticoid was used by Selye to refer to a group of adrenal steroids which exert a marked effect upon the metabolism of carbohydrates Mineralocorticoids are complementary to glucocorticoids and exert their effects upon the regulation of mineral metabolism It is convenient to refer to glucocorticoids and mineralocorticoids together as corticosteroids However the terms glucocorticoid and mineralocorticoid are not entirely specific in that certain members of each group show some of the actions characteristic of the other group Furthermore the use of the word corticosteroid in this sense is not universal and sometimes it is loosely applied to any steroid secreted by the adrenal cortex It will be shown later that glucocorticoid and mineralocorticoid molecules share certain structural characteristics which make the term corticosteroid a convenient way of referring to them both as a group

The role played by the adrenal cortex in regulating mineral and carbohydrate metabolism has so far overshadowed the part which the gland plays in the function of reproduction that discussions of cortical physiology are apt to centre about the former to the exclusion of the latter Some writers for example have suggested that hydrocortisone is the only cortical hormone The importance of the adrenal sex hormones however has become increasingly evident in recent years and they merit fuller treatment than they usually receive

because it also possesses glucocorticoid properties and is oxygenated at C_{11}

Corticosterone was the first compound of this group to be identified but its physiological activity is weak

11-deoxycorticosterone is an active mineralocorticoid which was synthesised relatively easily it therefore became of great clinical importance It is chiefly concerned with the metabolism of minerals and water

11-dehydrocorticosterone is remembered as the compound used to produce cortisone in 1948 [3]

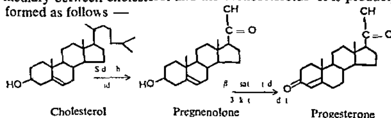
17 hydroxy 11-deoxycorticosterone is of little physiological importance while 17 hydroxy-11-dehydrocorticosterone is better known as cortisone or compound E

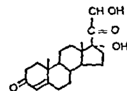
17 hydroxycorticosterone may eventually prove to be the true corticosteroid secreted by the human cortex [4,5] Its physiological actions are qualitatively identical with those of cortisone and it is generally known as hydrocortisone compound F or cortisol

Synthesis of Adrenocortical Steroids

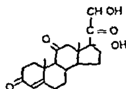
1 *SEX HORMONES* Very little is known about the synthesis of the adrenal androgens and of oestrone while progesterone is thought to be formed during the synthesis of corticosteroids It has been suggested that the first step in the synthesis of androgens involves the conversion of cholesterol to dehydroepiandrosterone (Fig 6)

2 *CORTICOSTEROIDS* It is generally believed that cholesterol and its esters are the source from which the glucocorticoids and mineralocorticoids are derived This concept is based upon experiments in which the isolated gland is perfused with solutions containing various steroid precursors[6] and upon the observation that ACTH causes a sharp fall in the level of serum cholesterol while at the same time causing the cortex to produce corticosteroids in greater quantity It has been further shown as the result of elaborate perfusion studies that progesterone is the key intermediary between cholesterol and the corticosteroids It is probably formed as follows —

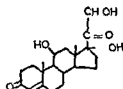




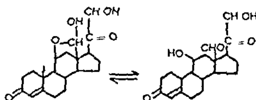
17 hydroxy
11 deoxycorticosterone
(Compound S)



17 hydroxy
11-dehydrocorticosterone
(Compound E)
Cortisone



17 hydroxycorticosterone
(Compound F)
Hydrocortisone



Aldosterone

The remaining 17 steroids are either incompletely identified chemically but are physiologically active (amorphous fraction) or they are inert (inert fraction)

The seven corticosteroids possess two structural characteristics in common The first is the α β unsaturated ketone group at C_3 i e —



By this is meant that in addition to a ketone group at C_3 (C = O) there exists a double bond (unsaturation) between the C_4 *and* C atoms which occupy positions in relation to the ketone *group which are referred to as* α *and* β *respectively The second* characteristic concerns the side chain attached to C_{17} This chain contains a ketone (C = O) at C_{20} and a hydroxyl group (OH) at C_{21} These two characteristics are essential for corticosteroid *activity*

In addition to these characteristics which distinguish the corticosteroids, it may be pointed out that *glucocorticoids* are oxygenated at C_{11} and are sometimes referred to as 11-oxysteroids *Mineralocorticoids on the other hand do not bear an oxygen atom* at C_{11} and may be referred to as 11 deoxycorticosteroids The important glucocorticoids are cortisone and hydrocortisone 11 deoxycorticosterone and aldosterone are the important mineralocorticoids *Aldosterone is somewhat exceptional in this respect*

Fig 6 Relation between the synthesis of corticosteroids and androgens This scheme represents a hypothesis accounting for the relationship between the synthesis of corticosteroids and that of cortical androgens It is doubtful whether interconversion between the upper 21-carbon atom and lower 19-carbon atom series takes place within the adrenal cortex The steps between progesterone and the corticosteroids are shown in detail in Fig 55

Fig 5 The synthesis of corticosteroids (*P M F Bishop*)

Fig 6 Relation between the synthesis of corticosteroids and androgens This scheme represents a hypothesis accounting for the relationship between the synthesis of corticosteroids and that of cortical androgens It is doubtful whether interconversion between the upper 21-carbon atom and lower 19-carbon atom series takes place within the adrenal cortex The steps between progesterone and the corticosteroids are shown in detail in Fig 55

When progesterone is perfused through the isolated gland 17 hydroxycorticosterone is produced (Fig 5) This requires three steps — 17 hydroxylation 21 hydroxylation and 11 hydroxylation It has been shown that once C_{11} bears a hydroxyl (OH) group it is no longer possible to add a hydroxyl group at C_{17} Therefore in the conversion of progesterone to 17 hydroxycorticosterone either 17 hydroxylation occurs first followed by 11 hydroxylation or 11-hydroxylation occurs before 17 hydroxylation If progesterone undergoes 21 hydroxylation deoxycorticosterone is formed and this can undergo 11 hydroxylation to give corticosterone (Fig 3) Finally 17 hydroxycorticosterone and corticosterone can each undergo 11 oxidation with the production of 17 hydroxy 11 dehydrocorticosterone (cortisone) and 11-dehydrocorticosterone respectively (Fig 5)

These reactions are thought to be controlled by enzyme systems (11 hydroxylase 21 hydroxylase 17 hydroxylase and 11-oxidase) It seems likely that competition for these enzymes may take place and that the resulting chemical equilibrium may determine the predominant activity of the gland at any given time For example compound S and DOC will compete for 11 hydroxylase (Fig 5) and upon the outcome of this competition will depend the proportion of hydrocortisone to corticosterone produced

This conception of enzyme competition may eventually help to explain the response of the cortex to the needs of the moment in terms of glucocorticoid or mineralocorticoid production It may also explain the one sided hormone production encountered in certain disease states

In addition to cholesterol ascorbic acid is present in the cortex in physiological quantities The role of ascorbic acid in steroid synthesis remains uncertain but its importance is revealed by the fall in cortical ascorbic acid which follows injections of ACTH This effect of ACTH is used as a means of quantitative estimation of this hormone in blood

Histology of the Cortex in Relation to Function

The striking arrangement of the cortical cells into well defined zones has suggested two theories concerning the functional significance of these layers Some workers[7,8] believe that the zona glomerulosa consists of immature cells which gradually mature and in doing so sink deeper into the cortex so that the cells of the outer part of the zona fasciculata contain the precursors of the cortical steroids The inner layers of the zona fasciculata are re

sponsible for the synthesis of the hormones which are finally discharged *from the cells of the zona reticularis* In other words, the arrangement of the cells of the cortex is based upon a cycle of maturation and degeneration cells being continually replaced from the zona glomerulosa (Fig 4)

The second theory postulates that each zone is responsible for secreting different hormones in the following manner —

Zona glomerulosa ⟶ mineralocorticoids

Zona fasciculata and zona reticularis ⟶ glucocorticoids.

X zone or inner part of zona reticularis ⟶ androgens

Each of these theories is supported by experimental evidence and so far the question has not been decided in favour of either

Action of Adrenocortical Hormones

Although it has been possible to learn a great deal about adrenocortical physiology from the study of diseases involving the gland and from the clinical and experimental use of pure hormone preparations much still remains obscure Certain effects observed in disease or during experiments may not accurately reflect the behaviour of the gland under physiological conditions For example large doses of cortisone may alter the distribution of body fat causing the face and trunk to bear an excess of adipose tissue while the limbs remain thin This same pattern of obesity is seen in certain diseases of the adrenal cortex but the exact role played by the cortex in controlling the distribution of body fat in normal individuals is unknown

1 SEX HORMONES

(a) *OESTROGENS AND PROGESTERONE* There is no reason to suppose that the action of oestrone and progesterone secreted by the cortex differs in any way to the action of these hormones secreted elsewhere The significance of oestrone and progesterone in the male is at present obscure and no further reference to their actions will be made in this chapter

(b) *ANDROGENS* In the male the effect of adrenal androgens is obscured by the presence of testicular hormones The action of adrenal androgens has therefore been studied in eunuchs in women and in patients suffering from diseases of the adrenal cortex

1 *Pubic and Axillary Hair* The appearance of some pubic hair and axillary hair in untreated eunuchs (the pubic hair showing a distribution characteristic of the female) is generally taken to

indicate that adrenal androgens play some part in the distribution of body hair and may be largely responsible for the development of pubic hair in the female

2 *Genital Organs* It seems likely that adrenal androgens are *responsible for the development of the clitoris and the labia* majora When adrenocortical hyperplasia affects females before birth hypertrophy of the clitoris, together with deformities of the urethra and distal portion of the vagina are seen What part adrenal androgens play in the embryology of the female genital tract under physiological conditions is unknown

3 *Libido* Adrenal tumours in women may cause a variety of changes in the direction of the sexual instinct from complete suppression of libido to overt homosexuality Small boys similarly affected show the sexual behaviour of adult men It is worth mentioning that the somatic precocity seen in these diseases is frequently accompanied by a striking maturity of speech and manner However interesting as the study of these phenomena may be it is not possible to say how far the changes observed are related to cortical function in normal subjects

4 *Epiphysial Closure* Adrenal androgens play some part in skeletal maturation but the numerous factors involved in bringing about epiphysial closure are too complex to permit of an exact statement of the parts played by the various hormones concerned

5 *Muscular Strength* Like testicular androgens those derived from the cortex are protein anabolic and cause nitrogen retention Excessive muscular development and preternatural strength may be encountered in the presence of excess adrenal androgens

2 CORTICOSTEROIDS

A GLUCOCORTICOIDS Of all the adrenocortical hormones the glucocorticoids have the most important actions It is the lack of glucocorticoids which eventually causes adrenalectomised animals to perish and cortisone by itself appears to serve as adequate substitution therapy in patients deprived of all cortical tissues It may be that hydrocortisone is not only the chief glucocorticoid of the human cortex but that under physiological conditions it is also partly responsible for the mineralocorticoid activity of the gland

1 *Carbohydrate Metabolism* The action of glucocorticoids upon carbohydrate metabolism is fourfold

(i) Glucocorticoids depress the reabsorption of glucose from the renal tubules and therefore promote glycosuria

(ii) They bring about an increase in the destruction of protein and in its conversion to carbohydrate or rather it may be that they divert amino acids towards carbohydrate metabolism at the expense of protein synthesis

(iii) Glucocorticoids also cause an increase in the deposition of glycogen in the liver This action forms the basis of one method of estimating the concentration of glucocorticoids in blood or urine At the same time no increase in muscle glycogen results from the action of glucocorticoids which indicates that there is no increase in the use of carbohydrate

(iv) Glucocorticoids interfere with the utilization of sugar by the body The mechanism of this action is uncertain but it is probable that hydrocortisone inhibits hexokinase an enzyme which normally accelerates the phosphorylation of glucose This effect of hydrocortisone is opposed to the action of insulin which is to promote the activity of hexokinase This failure of phosphorylation means that an important source of energy is left unused by the tissue cells and in this way hydrocortisone can impede cell mitosis

The overall effect of glucocorticoids when present in excess is to produce hyperglycaemia (and glycosuria) of a type which is resistant to the action of insulin In addition glucocorticoids play some part in the absorption of carbohydrate from the alimentary tract since in their absence absorption is delayed.

2 *Protein Metabolism* Glucocorticoids are protein catabolic an effect which brings about widespread changes in the body tissues [a]

(i) Glucocorticoids bring about increased destruction of protein and the amino acids thereby released are in part converted to carbohydrate

(ii) Accompanying the increased catabolism of protein there occurs a negative nitrogen balance and increased urinary excretion of creatine and uric acid

(iii) This catabolism of protein involves the tissues themselves with the following results —

- a wasting of skeletal muscles
- b thinning of the epidermis
- c diminished cellularity of connective tissue with breakdown of the intercellular structures and of the epithelial cells

d delay in wound healing and suppression of the fundamental changes of inflammation (i e the appearance of fibroblasts polymorphonuclear cells and lymphocytes),

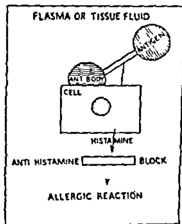
e osteoporosis due to loss of protein from the matrix of bone

3 *Fat Metabolism* Glucocorticoids cause a rise in the concentration of serum fat (triglyceride) and in large doses may lead to the appearance of adiposity, showing a characteristic distribution in which the face cervical region and abdomen are affected but the limbs are spared

4 *Electrolyte and Water Metabolism* The action of glucocorticoids upon the body metabolism of electrolytes and water is variable but in man they appear to cause retention of sodium and chloride while stimulating the excretion of potassium This action although qualitatively the same as that of mineralocorticoids is much less powerful than in the case of the latter hormones

5 *Membrane Permeability* Glucocorticoids depress the permeability of various membranes *e g synovial membranes* and oppose the spreading action of hyaluronidase Hyaluronidase is thought to release hyaluronic acid from such membranes and thereby increase their permeability glucocorticoids oppose the action of hyaluronidase

6 *Blood Cells* Cortisone causes lymphopaenia and depression of lympoid tissue It also causes a fall in the number of circulating eosinophils This last effect is used clinically in testing adrenocorti-



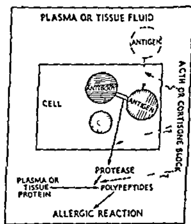


Fig 7 Diagrammatic illustration of two types of allergic reactions (*P M F Bishop*)

cal function and may result from lysis of eosinophils or more probably, the cells are sequestered in some reticulo-endothelial tissue

7 *Allergic Response* Glucocorticoids suppress the exaggerated inflammatory reaction which is characteristic of the allergic response Pickering[10] believes that there are two distinct varieties of allergic reactions (Fig 7)—the immediate response which involves the union of antigen and antibody on the cell surface with the immediate release of histamine and the delayed response which involves intracellular union of antigen and antibody with the subsequent release of an enzyme Immediate responses are prevented by antihistamine drugs whereas delayed responses (seen in asthma dermatitis rheumatoid arthritis etc) are prevented by cortisone

8 *Mental Changes* Cortisone is capable of producing a feeling of elation and in large doses it may bring about an exaggeration of personality trends leading eventually to insanity Changes seen in electroencephalograms of patients receiving cortisone include disturbances of alpha activity and the appearance of bursts of slow waves

9 *Gastric Activity* Glucocorticoids stimulate the secretion of hydrochloric acid and pepsin by the stomach

10 Glucocorticoids assist the body to remove a water load This property is used as a means of testing the capacity of the cortex to secrete glucocorticoids (the so-called Robinson Kepler Power test) The mechanism of this action upon water excretion remains obscure It may result from an increase in renal blood flow or by suppression of the secretion of antidiuretic hormone or by increase in the rate at which the tissues of the body inactivate this hormone

B MINERALOCORTICOIDS Deoxycorticosterone causes marked retention of sodium chloride and water at the same time it increases the urinary output of potassium and phosphorus by the direct action of the steroid upon the distal part of the renal tubules The activity of deoxycorticosterone is largely confined to the regulation of body electrolytes since it exerts no effect upon carbohydrate metabolism and shares none of the other actions of glucocorticoids *Whether the primary effect of the hormone is* upon sodium excretion—the other effects being secondary to this or whether it exerts some direct action on the metabolism of potassium and water remains doubtful

It had long been known that the amorphous fraction (see page 28) exhibited a powerful mineralocorticoid action but it was not

until 1952 that this was shown to be due to the presence of aldos terone, formerly known as electrocortin since obtained in crystal line form and identified chemically [11] The salt retaining properties of aldosterone are many times stronger than those of deoxycorti costerone In addition to its mineralocorticoid properties aldoster one is capable of protecting experimental animals against stress, shows some glucocorticoid action and will bring about eosinopaenia[11 12 13] What part aldosterone and deoxycorticosterone play separately or together, in the function of the adrenal cortex under physiological conditions is uncertain Some authors believe they are both important others hold that aldosterone is the true mineralocorticoid of the cortex and that deoxycorticosterone is simply an intermediary compound formed during the synthesis of other steroids

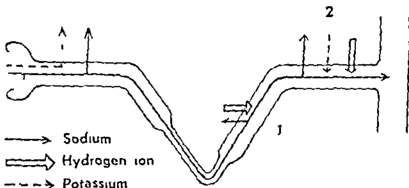


Fig 8 The renal excretion of sodium and potassium The site of action of *mineralocorticoids is indicated by 1 and 2 At 1 the hormones influence the* exchange of sodium and hydrogen ion while at 2 they control the exchange of sodium and potassium and possibly the exchange of sodium and hydrogen ions

Recent experiments have served to extend our knowledge of the mechanism by which mineralocorticoids regulate the excretion of sodium and potassium The potassium of the glomerular fil trate is entirely reabsorbed in the proximal part of the tubule while the potassium of the urine is derived exclusively from the cells of the distal part of the tubule This distal secretion of pot is sium involves exchange with sodium, which is reabsorbed [7] Sodium is also reabsorbed from the proximal convoluted tubule and there is good evidence to show that reabsorption of sodium in exchange for hydrion also takes place in the ascending limb of the loop of Henle (Fig 8) It has been suggested as the result of experiments using isotopically labelled potassium that the action

of mineralocorticoids in increasing the reabsorption of sodium is exerted at the site of sodium hydrion exchange in the ascending limb of the loop and at the site of sodium potassium exchange in the distal convoluted tubule (Fig 8) These remarks imply that all the potassium secreted by the tubules is exchanged for sodium absorbed from the tubular fluid [7] It can therefore be seen that the tubular secretion of potassium is limited by the availability of sodium at the site of this cation-exchange This conception helps to explain the influence of sodium excretion upon that of potassium

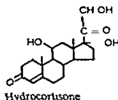
In contrast to glucocorticoids mineralocorticoid hormones have been shown to exert a prophlogistic action that is to say these hormones stimulate the proliferative reactivity or inflammatory potential of connective tissue The significance of this action is unknown

Synthetic Adrenocortical Hormones

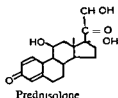
Certain changes in the chemistry of adrenal steroids have been shown to alter their physiological properties in a remarkable way

1 *Halogenation* The admission of chlorine or fluorine into the molecules of cortisone or hydrocortisone at C_9 has been shown to enhance their mineralocorticoid activity without loss of glucocorticoid properties Such halogenated glucocorticoids are many times stronger than deoxycorticosterone in their effects upon mineral metabolism

2 *Prednisolone* (Metacortandralone) and *Prednisone* (Metacortandracin) These compounds are synthetic crystalline steroids which possess hormonal activity Prednisolone is identical with hydrocortisone except for a double bond between C_1 and C_2 in the A ring and prednisone is identical with cortisone except for the same change These compounds are effective by mouth and show great anti rheumatic and anti inflammatory properties while at the same time they are less apt to induce salt retention than hydrocortisone and cortisone



Hydrocortisone



Prednisolone

3 2 *Methylcorticosteroids* The 2 methyl derivatives of cortisone and hydrocortisone show considerable activity in tests of sodium retention in fact 2 methyl 9α fluorohydrocortisone is more active in this respect than aldosterone [8]

Amphenone In 1950 a substituted deoxybenzoin was synthesised and named amphenone This compound which is not a steroid depresses the cortical production of steroids probably by a direct action upon the gland rendering it less responsive to the stimulating effect of ACTH

Adrenal Cortex and Renal Function [24, 25]

It has been suggested that the fundamental action of the adrenal cortex upon mineral and water metabolism is due to a direct action upon the tubules of the kidney This theory is however very difficult to confirm The results obtained by the application of the orthodox methods of studying the various phases of renal function in the presence of abnormal cortical activity have not given uniform results In animals subjected to total adrenalectomy and kept alive by means of cortisone studies of renal function have been made during the administration of cortisone and again after the drug has been withheld These investigations have suggested that the following changes occur after adrenalectomy —

1 Renal blood flow It is sometimes possible to demonstrate a fall in renal blood flow following adrenalectomy

2 Glomerular filtration Probably as the result of a fall in renal blood flow adrenalectomy produces a fall in the glomerular filtration rate

3 Tubular function In animals there appears to be an increased tubular excretion of sodium and a fall in potassium excretion

Miscellaneous Functions of the Adrenal Cortex

The adrenal cortex is concerned in some way with the production of the pigment melanin It has been suggested that corticosteroids inhibit the secretion of melanocyte stimulating hormone by the pituitary In addition glucocorticoids depress the production of compounds containing the sulphydryl (SH) group The latter compounds in turn inhibit the production of melanin The first of these observations may explain the occurrence of excessive pigmentation of the skin in diseases which involve lack of adrenocortical function

It is known that the cortex plays some part in the growth of young animals in the regulation of blood pressure and in the capacity of skeletal muscle to contract efficiently These miscellaneous effects appear to result indirectly from other actions of the gland Finally evidence has recently been produced for the existence of a sodium losing hormone secreted by the cortex [5] This evidence has been derived largely from studies of patients suffering from abnormal cortical function and is at present inconclusive

The Control of Adrenocortical Activity

The isolated adrenal gland is capable of some secretory activity However, under physiological conditions within the body the requirements of adrenocortical steroids vary so much from hour to hour and even from minute to minute that elaborate mechanisms exist which enable the gland to meet these variations

1 *SEX HORMONES* The control of the secretion of sex hormones has not been studied as fully as that of corticosteroids However ACTH can certainly stimulate the production of cortical androgens The details of this action are not understood but a hypothetical scheme to explain the relationship between the synthesis of androgens and of corticosteroids is shown in Figure 6

It has been suggested that ACTH stimulates the conversion of cholesterol to dehydroepiandrosterone and in this way promotes the synthesis of adrenal androgens While this attractive hypothesis is in harmony with the effect of ACTH upon the production of corticosteroids (see below) it remains unproven (Fig 6)

2 *CORTICOSTEROIDS* *Adenohypophysis* The anterior pituitary gland influences the cortex by secreting the adrenocorticotrophic hormone (ACTH or corticotrophin) Administration of ACTH to man and animals brings about hypertrophy of the adrenal cortex an increase in the concentration of cortical hormones in the adrenal vein and a fall in the cortical content of steroid precursors (cholesterol) and of ascorbic acid The fundamental action of ACTH appears to be concerned with the conversion of cholesterol to pregnenolone and it may be that the rate of this conversion sets the pace and direction of adrenocortical activity [6]

ACTH undoubtedly increases the production of glucocorticoids In the case of mineralocorticoids there appears to be some difference from one species to another and it remains uncertain to what extent (if any) ACTH can stimulate the production of

deoxycorticosterone and aldosterone in man Certainly aldosterone has been found in the adrenal vein blood of hypophysectomised dogs and can therefore be secreted in the absence of ACTH

STRESS Stress has been defined as any condition which increases the metabolic demands of the whole body or of some of its organs It is in effect a term applied to such noxious stimuli as cold heat trauma infection anaesthesia anoxia toxins and psychological stress It is known that exposure to such stimuli evokes a well marked response from the adrenal cortex and further that this response acts as a protective mechanism against the particular stress applied The response on the part of the cortex is brought about by a shift in pituitary hormone production away from gonadotrophic hormones in favour of ACTH[16] This increased production of ACTH is brought about in several ways

In the first place stress accelerates the rate at which adrenocortical hormones are used by the tissues of the body and in this way lowers their concentration in the blood This fall of the concentration of these hormones in the blood stimulates the production of ACTH by causing the direction of pituitary activity to shift away from the production of other trophic hormones towards the formation and release of ACTH This mechanism is referred to as the Sayers tissue utilization hypothesis[34]

Secondly stress causes the adrenal medulla to discharge adrenaline which in turn causes the pituitary to release ACTH This mechanism is called the Long adrenaline hypothesis[1]

Finally Harris has produced convincing evidence to show that stress may affect the production of ACTH by acting upon the hypothalamus which is in turn capable of stimulating increased production of ACTH by the pituitary This mechanism is called the Harris hypothalamic hypothesis[16]

How these three mechanisms share the management of adrenocortical function is uncertain It has been suggested that the reciprocity between serum corticosteroid levels and ACTH secretion may account for a steady balance during conditions of relative stability Upon this is superimposed the hypothalamic control which is responsible for day to day variations in cortical activity while all three mechanisms come into play under conditions of acute stress Harris believes that there exists a basal level of hypothalamic drive under resting conditions which is increased under the influence of stress

OTHER FACTORS Perfusion studies have stressed the importance of at least two other substances (adenosine triphosphate

and potassium) in controlling the rate of hormone production by the isolated gland

Adenosine Triphosphate This compound produces a sharp burst of steroid hormone secretion by the cortex The gland is known to take up and use phosphates[17] and this behaviour may be due to the action of ACTH

Potassium During perfusion experiments an increase in the potassium content of the perfusing fluid brings about an increase in corticosteroid production The special interest in this response lies in the fact that such a mechanism could control the mineralocorticoid secretion of the cortex without the intervention of the pituitary gland Such a concept would accord well with the observation that in some animals at least the secretion of mineralocorticoids is independent of pituitary control However changes of potassium concentration in the blood may only represent an emergency mechanism which is not responsible for governing the secretion of mineralocorticoids within physiological levels

Autonomous Activity Experimental evidence in animals suggests that there exists an inherent cortical activity which provides a basic hormone output in the absence of the mechanisms of control already described

While the secretion of aldosterone appears to enjoy a certain freedom from the control of the adenohypophysis a fall in extracellular fluid volume stimulates the rate of its production It is more difficult to disentangle the effects of changes in serum potassium and sodium but it seems likely that a rise in body potassium stimulates the secretion of aldosterone while a fall has the opposite effect By and large, changes in body sodium produce the opposite effects Recent studies[25] suggest that the effect of changes in body potassium are mediated by way of a direct influence upon the cortex whereas changes in body sodium operate by way of associated fluctuations in fluid balance Experiments which attempt to separate these different factors often produce highly artificial conditions and must be interpreted with caution

Stress is believed to cause some increase in the rate of secretion of aldosterone while decapitation causes a fall in the adrenal vein content of this hormone Some workers believe that the latter observation points to some cerebral factor (possibly hypothalamic) in the control of a dosterone secretion [8]

Urinary Excretory Products of Adrenocortical Hormones

Little is known of the fate of adrenocortical hormones This is a natural consequence of the uncertainty which surrounds the

deoxycorticosterone and aldosterone in man Certainly aldosterone has been found in the adrenal vein blood of hypophysectomised dogs and can therefore be secreted in the absence of ACTH

STRESS Stress has been defined as any condition which increases the metabolic demands of the whole body or of some of its organs It is in effect a term applied to such noxious stimuli as cold heat trauma infection anaesthesia anoxia toxins and psychological stress It is known that exposure to such stimuli evokes a well marked response from the adrenal cortex and further that this response acts as a protective mechanism against the particular stress applied The response on the part of the cortex is brought about by a shift in pituitary hormone production away from gonadotrophic hormones in favour of ACTH[13] This increased production of ACTH is brought about in several ways

In the first place stress accelerates the rate at which adrenocortical hormones are used by the tissues of the body and in this way lowers their concentration in the blood This fall of the concentration of these hormones in the blood stimulates the production of ACTH by causing the direction of pituitary activity to shift away from the production of other trophic hormones towards the formation and release of ACTH This mechanism is referred to as the Sayers tissue utilization hypothesis [14]

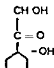
Secondly stress causes the adrenal medulla to discharge adrenaline which in turn causes the pituitary to release ACTH This mechanism is called the Long adrenaline hypothesis [15]

Finally Harris has produced convincing evidence to show that stress may affect the production of ACTH by acting upon the hypothalamus which is in turn capable of stimulating increased production of ACTH by the pituitary This mechanism is called the Harris hypothalamic hypothesis [10]

How these three mechanisms share the management of adrenocortical function is uncertain It has been suggested that the reciprocity between serum corticosteroid levels and ACTH secretion may account for a steady balance during conditions of relative stability Upon this is superimposed the hypothalamic control which is responsible for day to day variations in cortical activity while all three mechanisms come into play under conditions of acute stress Harris believes that there exists a basal level of hypothalamic drive under resting conditions which is increased under the influence of stress

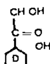
OTHER FACTORS Perfusion studies have stressed the importance of at least two other substances (adenosine triphosphate

(i) *Porter Silber Method* A characteristic yellow colour appears as the result of reaction between phenylhydrazine hydrochloride and steroids which have a 17 21 dihydroxy 20 ketone grouping i e

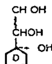


The intensity of this yellow colour can be measured and a number of modifications of the method are in use The range of normal for this method varies according to the details of technique It is common practice to refer to the substances estimated in this way as 17 hydroxycorticoids or 17 hydroxysteroids in contrast to 17 ketosteroids but this implies a specificity for the test which it may not possess

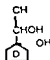
(ii) *Oxidation Method* The side chain at C_{17} can be removed from certain steroids by sodium bismuthate to give 17 ketosteroids These steroids include those bearing the following groups —



(a)



(b)



(c)

Steroids in the blood or urine bearing these groups are thought to be by products of glucocorticoid metabolism The increase in 17 ketosteroid content of urine occasioned by this process of oxidisation therefore gives some indirect measure of the concentration of glucocorticoids in blood Only steroids bearing the group shown as (a) above are estimated by the Porter Silber method which means that the oxidation method gives higher results than those obtained from the Porter Silber technique

Recent modifications of this method include removal of pre existing 17 ketosteroids by means of sodium borohydride

While each laboratory must establish its own normal range for these estimations normal adults show levels for the Porter Silber method of between 2 and 10 mgm per 24 hours and for the oxidation methods of between 8 and 20 mgm per 24 hours

2 *BIOASSAY*

(i) *Venning Method* [9] This method measures the glucocorticoid content of urine and depends upon the ability of 11 oxygen

nature and number of these steroids in the blood It appears likely that a proportion of the cortical hormones is excreted in the urine unchanged but that this proportion is small The bulk of cortical steroids undergoes a series of chemical changes in the course of their physiological activities and the end products of these chemical changes appear in the urine Present day knowledge of these excretory products is still imperfect but appears to offer a valuable reflection of adrenocortical activity

ANDROGENS Among the six adrenal androgens mentioned above five will be seen to possess a ketone (C = O) group at C_{17} In this form they appear in the urine i e as 17 ketosteroids the rest of the molecule being in some cases altered during their metabolism In the urine 17 ketosteroids can be relatively easily and accurately estimated by a colorimetric method All 17 ketosteroids are not however the end products of adrenal androgen metabolism In the male about one third of the total 17 ketosteroid output is derived from testicular androgens In both sexes certain members of the pregnane series of steroids which are not themselves androgens but which represent intermediary compounds in the synthesis or end products of the metabolism of non androgenic steroids can undergo oxidation of the side chain at C_{17} and thereby contribute towards the total 17 ketosteroid output When the term 17 ketosteroid is used without qualification it refers to neutral 17 ketosteroids this excludes certain oestrogens and other steroids which have a phenolic structure in the A ring of the nucleus Normal adult males excrete between 8 and 20 mgm of neutral 17 ketosteroids in 24 hours females between 4 and 12 mgm

GLUCOCORTICOIDS AND MINERALOCORTICOIDS The measurement of urinary products of corticosteroids is fraught with the same difficulties as those encountered in the case of androgens The distinction between glucocorticoids and mineralocorticoids is confused by the fact that 11 deoxycorticosterone is excreted in part as pregnanediol and in part as corticosterone

1 *CHEMICAL ASSAYS* Two main methods are in current use (i) The Porter Silber colour reaction and

(ii) Methods which involve the oxidation of the metabolic products of glucocorticoids to 17 ketosteroids and subsequent measurement of these compounds by the colorimetric methods mentioned above The difference between the 17 ketosteroid estimations before and after oxidation gives a measure of glucocorticoid metabolites

In a normal individual at least one of the four successive hourly specimens collected in the morning exceeds in volume the total nocturnal output In the presence of deficient glucocorticoid secretion the ingestion of water fails to stimulate this diuresis and consequently the nocturnal volume exceeds that of each morning specimen

The mechanism of this test is somewhat uncertain An abnormal response may result from alterations in renal blood flow associated with disease of the adrenal cortex or to changes in the release of antidiuretic hormone from the neurohypophysis

c) *Urinary Assays* Either by the Venning method or by one of the indirect chemical tests already mentioned it is possible to measure (somewhat imperfectly) the excretion of glucocorticoids

3 *MINERALOCORTICOID SECRETION* Although tests involving the use of low salt diets were once used to assess the ability of the cortex to secrete mineralocorticoids they now find no place in clinical medicine because they are dangerous and are rarely required

Diseases of the Adrenal Cortex

Diseases of the adrenal cortex in man have been closely studied and because they illustrate certain aspects of cortical function the more important examples can be mentioned

HYPOFUNCTION Addison s Disease This condition represents a failure on the part of the adrenal cortex to secrete adequate quantities of steroid hormones It may result from one of a number of pathological processes affecting the gland Deficiency of glucocorticoids is shown by the great susceptibility of the patients to stress Failure of mineralocorticoid secretion leads to a fall in serum sodium and a rise in potassium Such patients show a low resistance to infection and readily become dehydrated The appearance of areas of skin pigmentation due to the deposition of melanin is a highly characteristic feature of the disease

HYPERFUNCTION 1 Adrenocortical tumours may produce a picture of excess secretion of androgens glucocorticoids and mineralocorticoids Female patients show evidence of masculinization e g amenorrhoea excess body hair of male distribution and hypertrophy of the clitoris Adiposity affecting the face and trunk is seen and diabetes together with atrophy of the epidermis and loss of muscular tissue are features of the condition

ated steroids to deposit glycogen in the liver of the fasting adrena lectomised mouse

(ii) *Cortin Assay* An ill defined mixture of glucocorticoids and mineralocorticoids can be estimated by a number of bioassay methods The substances measured in this way are referred to as cortin

Tests of Adrenocortical Function

Ideally, tests of adrenocortical function would consist of chemi cal assays of some or all of the important cortical steroids in blood or urine Present day tests fall far short of this ideal In the clini cal assessment of adrenocortical function it is important to bear in mind the three important groups of hormones secreted by the cortex (androgens glucocorticoids and mineralocorticoids) Dif ferent tests involve different groups of hormones and interpretation of a given test involves the consideration of which hormone or group of hormones is being subjected to measurement

1 *ANDROGEN SECRETION* Estimation of the total neutral 17 ketosteroid excretion gives some indirect measure of the androgenic activity of the cortex and gross variations from the normal are significant

Efforts have been made to measure the excretory products of adrenal androgens as distinct from testicular androgens and non androgenic steroids For example it is possible to measure the percentage of 17 ketosteroids in the urine which *constitute the* β fraction By this is meant those 17 ketosteroids in which the hydroxyl group (OH) at C_3 lies in the same plane as the methyl group at C_{10} The β fraction normally constitutes about 15 per cent of the total 17 ketosteroid output and is chiefly de rived from the adrenal androgens

2 *GLUCOCORTICOID SECRETION* a) *Tests of eosinopaenia production*[21 2] The capacity of the adrenal cortex to secrete glucocorticoids may be measured by studying the fall in the number of circulating eosinophils resulting from an injection of ACTH The ACTH stimulates the cortex to produce glu cocorticoids which in turn cause a fall in circulating eosinophils

b) *Kepler Test* This test is a useful indirect method of assessing glucocorticoid secretion and of its many modifications that described by Kepler Robinson and Power [3] is widely used This consists of measuring the hourly urinary volumes excreted after ingestion of a calculated amount of water early in the morning

CHAPTER III

THE THYROID GLAND

Introduction

The thyroid gland is present in all vertebrates and during its development possesses a duct like appendage which has suggested to some workers that the gland once delivered an external secretion into the pharynx by means of this duct However no species of animal is known in which such an exocrine function occurs and in vertebrate physiology the gland plays a purely endocrine role In both warm blooded and cold blooded animals the thyroid gland shows seasonal variations in function but in the former its activity is greater in cold weather while in the latter the reverse is true

Embryology

The thyroid gland is derived from the midventral floor of the pharynx By the end of the fourth week a small hollow sac joined to the pharynx by a tubular duct has developed (Fig 9) during the sixth week this thyroglossal duct atrophies Meanwhile the thyroid gland loses its central cavity and adopts a bilobed form (Fig 9) During the eighth week discontinuous cavities appear

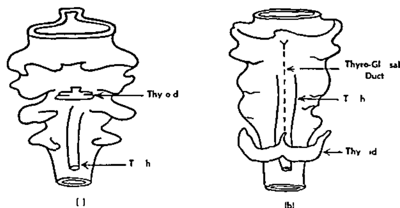


Fig 9 The development of the thyroid gland (*a*) at the sixth week (*b*) at the seventh week

2 *Adrenocortical hyperplasia before birth* When adrenocortical hyperplasia occurs before birth it may interfere with the development of the female genital tract so that at birth the sex of the affected individual may be indeterminate This condition is referred to as female pseudohermaphroditism

REFERENCES

The following list contains some of the outstanding papers dealing with various aspects of adrenocortical physiology An exhaustive list of references will be found in Diseases of the Endocrine Glands by L J Soffer Lea & Febiger Philadelphia 1956 The recent literature on this subject is fully and critically reviewed in Recent Advances in Endocrinology by P M F Bishop J & A Churchill London 1954

1 Graham L S Cancer 6 149 1953
2 Cope O and Raker J W N England J Med 253 119 1955
3 Sarett L H J Amer Chem Soc 70 1454 1948
4 Reich H Nelson D H and Zaffaroni A J Biol Chem 187 411 1950
5 Nelson D H Reich H and Samuels L T J Clin Endocrinol 10 810 1950
6 Hechter O et al Recent Progress in Hormone Research Vol vi p 215 Academic Press New York 1951
7 Ingle D J Pituitary—Adrenal Function p 49 Amer Ass Advancement of Science Washington 1950
8 Greep R O Deane H W Endocrinology 45 42 1949
9 Browne J S L Brit Med J ii 191 1951
10 Pickering G W Brit Med J i 1207 1952
11 Simpson S A et al Experentia 10 132 1954
12 Spiers R S Simpson S A Tait J F Endocrinology 55 233 1954
13 Gross F Gysel H Acta Endocrinol 15 199 1954
14 Sayers G Physiol Rev 30 241 1950
15 Long C N H Pituitary—Adrenal Function p 24 Amer Ass Advancement of Science Washington 1950
16 Harris G W Brit Med J ii 627 1951
17 Reiss M Halkeston J M J Endocrinol 6 369 1950
18 Talbot N B et al J Biol Chem 160 535 1945
19 Heard R D H Sobel H J Biol Chem 165 687 1946
20 Venning E H Kazmin V E Bell J C Endocrinology 38 79 1946
21 *Renold A E New Engl J Med 244 796 1951*
22 Jenkins D et al Amer J Med 18 3 1955
23 Bishop P M F Recent Advances in Endocrinology p 77 Livingstone London 1954
24 Talbot J H et al J Clin Invest 21 107 1942
25 Harrison H E Darrow D C Am J Physiol 125 631 1940
26 Selye H J Clin Endocrinol 6 117 1946
27 Morel F Aldosterone p 180 J & A Churchill Ltd London 1958
28 Bayliss R I S Modern Trends in Endocrinology Butterworth & Co London 1958

either directly into the blood stream or into the follicular lumen In the second event the secretion will pass back through the cell in which it was originally secreted on its way to the blood stream (Fig 12) The mitochondria [3] of the thyroid cell increase in number when the gland is active and this increase seems to be related to the formation of colloid rather than to the release of hormone into the blood Hypertrophy of the Golgi apparatus on the other hand is associated with increase in the rate of release of hormone into the circulation[4]

Ectopic Thyroid Tissue[1]

Ectopic thyroid tissue may develop anywhere along the path of descent of the thyroid gland Consequently such tissue may be found within the tongue itself lingual and sublingual thyroid tis sue may occur in the absence of a normal thyroid gland Some times active glandular tissue is found in the mediastinum as far down as the diaphragm

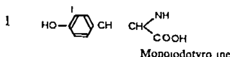
Function

The function of the thyroid gland is to produce a colloid material containing a hormone which is capable of stimulating the rate of metabolism of the tissues according to the needs of the body

Chemistry of Thyroid Secretion

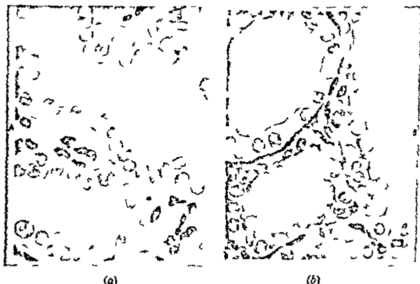
The colloid of the thyroid gland contains two proteins Of these the first is a nucleoprotein derived from the destruction of cell protoplasm—it is iodine free and physiologically inert The second is a globulin which contains iodine when this is given to experimental animals it is capable of substituting for the intact gland This second protein is a true secretory product of the thyroid cell and is called iodothyroglobulin (thyroglobulin for short)

The thyroid gland contains at least seven compounds whose molecules bear iodine

1 

Monoiodotyrosine

within the gland These represent the follicles of the adult gland and soon come to contain a colloid substance At the end of the fourth month this formation of follicles comes to an end and thereafter new follicles are formed by the budding and division of those already in existence



(a) (b)

Fig 10 The histology of the thyroid gland (a) active phase (b) quiescent phase

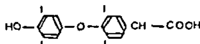
Histology

The characteristic feature of the thyroid gland is the follicle—a roughly spherical structure lined by epithelium and containing colloid (Fig 10) This colloid is a homogeneous substance rich in iodine and in the fresh state it is clear when fixed it stains with acid dyes The lining epithelium is cuboidal and rests directly upon the vascular connective tissue between the follicles, without the intervention of a basement membrane

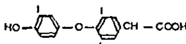
The epithelium varies in appearance with the state of activity of the gland Generally speaking the cells are low when the gland is resting and tall when it is active A low epithelium is usually associated with a large follicular lumen and an increase in colloid content Tall columnar epithelium, on the other hand is usually to be seen with small follicles and consequent folding of the redundant epithelium upon itself The thyroid cell has been subjected to close scrutiny which has disclosed certain features of interest Apparently the cells lining the follicle have the power to secrete

Nature of Thyroid Hormone

The thyroid gland secretes more than one hormone Thyroxine 3 5 3 triiodothyronine 3 3 5 triiodothyronine and 3 3 -diiodothyronine are all released from the gland into the blood stream where they become attached to the serum proteins In addition the acetic acid derivatives tetraiodothyroacetic acid (TETRAC) and triiodothyroacetic acid (TRIAC) have been isolated from certain tissues of the rat and may represent either metabolic products of thyroid hormones or even additional hormones[24 5] So far however the two acetic acid derivatives have not been found in blood so that only the four thyronines can be regarded as true thyroid hormones at the present time[5]



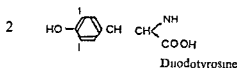
3.5 3 5 Tetraiodothyroacetic acid (TETRAC



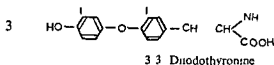
3 5 3 Triiodothyroacetic acid (TRIAC)

In all its physiological actions 3 5 3 triiodothyronine is more active weight for weight, than thyroxine This observation has suggested that 3 5 3 triiodothyronine is the true thyroid hormone or at any rate the most important hormone However it is now generally agreed that thyroxine is the principal thyroid hormone and that although other iodinated amino acids are secreted by the gland and released into the circulation their importance as hormones is slight beside that of thyroxine The great interest aroused by TETRAC and TRIAC lies in the fact that these substances show qualitative differences in their activity For example TRIAC is more effective than thyroxine or triiodothyronine in promoting metamorphosis of amphibia but is weak in preventing goitre and in stimulating the rate of oxygen consumption in the rat[23]

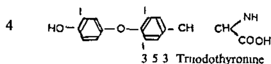
In blood the thyroid hormones are chiefly associated with the albumin and α globulin fractions of serum protein although the nature of this association is not known Some workers have suggested that thyroxine and triiodothyronine may exist in the blood stream in peptide combination forming a polypeptide molecule All that can be stated with certainty at present is that while the colloid contains thyroglobulin some fractions of this molecule in

2


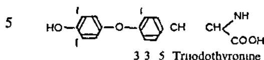
Diiodotyrosine

3


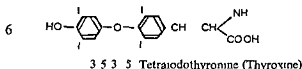
3 3 Diiodothyronine

4


3 5 3 Triiodothyronine

5


3 3 5 Triiodothyronine

6


3 5 3 5 Tetraiodothyronine (Thyroxine)

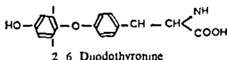
7 Thyroglobulin

Of these seven compounds the first six are amino acids Thyroglobulin is a large protein molecule with a molecular weight of about 700 000 The composition of thyroglobulin is somewhat variable but it contains up to 1 per cent of its weight of iodine

The significance of the iodinated amino acids other than thyroxine remains disputed they may be true hormones they may be precursors of thyroxine formed during the synthesis of this hormone or they may be derived from thyroxine as the result of metabolic changes brought about by the tissues The last of these possibilities has been elaborated into an attractive hypothesis which suggests that thyroxine is largely inactive except as a precursor of other iodinated amino acids The rate of removal of iodine from thyroxine which gives rise to these less iodinated compounds (triiodothyronines triiodothyroacetic acid and diiodothyronine) appears to depend upon the concentration of thyroxine in blood—the greater the concentration of thyroxine the more rapid the deiodination

(iii) The hydroxyl group (OH) must occupy the 4 position on the second ring If it be moved to another position the compound loses its physiological activity

(iv) The iodine atoms must occupy 3 5 positions the 2 6 analogues actually show thyroxine inhibiting properties e g —



2 6 Diiodothyronine

(v) The side chain $-CH_2-\underset{NH_2}{CH}-COOH$ is not essential for physiological activity This is shown by the action of acetic acid derivatives (see page 55)

Thyroxine is optically active and the naturally occurring (–) thyroxine is more active weight for weight than the (+) or racemic (±) isomers but no qualitative differences can be detected in the actions of these three substances

The synthesis of thyroid hormone requires an adequate supply of iodine and the circulation of iodine in nature is important in providing a source of this element to vertebrate animals

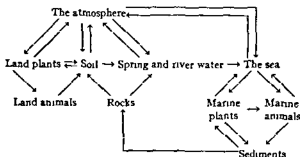


Fig 11 The iodine cycle in nature (*Best and Taylor*)

IODINE METABOLISM

Source Iodine occurs in soil whence it is relentlessly extracted by springs and rivers which convey the iodine to the ocean In this way sea water comes to contain an inexhaustible supply of iodine and Figure 11 indicates the exchange of iodine between land and sea The further away from the sea and the more mountainous the country the lower the concentration of iodine in food and water

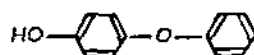
the form of iodinated amino acids are released into the blood stream, where they exist in a form which is described as protein bound

The globin protein responsible for binding the greater part of the thyroxine in plasma is called thyroxine binding protein (TBP) Electrophoretic studies have shown that TBP appears just ahead of alpha$_2$ globulin Thyroxine binds with this protein more firmly than triiodothyronine and can displace the latter from such binding TBP is present in plasma in a concentration which is greater than that required to bind normal concentrations of thyroxine with the result that the addition of small quantities of thyroxine (less than 0 1 mg /ml) to plasma causes the added hormone to be bound to TBP Further addition of thyroxine causes binding with all serum protein fractions albumin being the most important of these secondary carriers Studies performed upon blood taken from patients who do not possess thyroid tissue indicate that the thyroxine binding capacity of TBP is approximately 0 4 mg /ml and that about one third of this capacity is used in normal subjects During pregnancy the thyroxine binding capacity of TBP greatly increases from about the twenty first day after ovulation A similar increase in thyroxine binding capacity is seen after the administration of oestrogens and it has been suggested that these hormones are responsible for the changes described during pregnancy

Hydrolysis of thyroglobulin reveals that some 60 per cent of its iodine is present as iodotyrosines (15 per cent as monoiodotyrosine and 45 per cent as diiodotyrosine) and 30 per cent as iodothyronines Very little iodotyrosine is found free within the gland and none in the blood this is due to the rapidity with which these compounds lose iodine as soon as they are released from the thyroglobulin molecule

Certain changes in the chemistry of thyroxine have been shown to abolish its thyroid like action others to bring about a great decrease in its potency without qualitative change while yet other changes do not cause such striking differences in its activity [5] (The system used in numbering the atoms in the thyronine nucleus molecule is given on page 17)

(i) The physiological activity of thyroid hormone requires the nucleus



(ii) The introduction of halogens other than iodine causes a great decline in potency if more than one atom of iodine be replaced in this way

cells of its follicles Probably only inorganic iodide (free from serum protein) is selectively collected and the rate of uptake amounts to between 2 and 4 per cent per hour of the iodide present in the blood and body fluids[8] The trapping of iodide begins as soon as it enters the blood and the oxidation of trapped iodide to free iodine is performed with the same speed This process of oxidation is controlled by one or more enzymes and requires a considerable output of energy

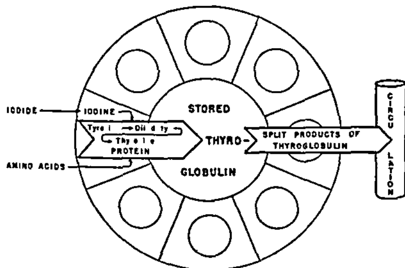


Fig 12 Diagram showing the synthesis of thyroid hormone The gland takes up iodide and converts it to iodine At the same time the thyroid removes amino acids (including tyrosine) from the circulation from these it constructs the protein of the thyroid Within the protein molecule tyrosine is iodinated and two molecules of diiodotyrosine are condensed to form one of thyroxine The last step may take place within the thyroid cell or in the follicle Thyroglobulin is broken down by proteolytic enzymes and the smaller molecules which result diffuse out of the gland (*J H Means*)

The second step in hormone production is the conversion of free iodine into protein bound iodine (Fig 12) Tyrosine is present in the thyroid gland and is iodinated within the cells of the follicle Actually the process takes place within the thyroglobulin molecule which contains tyrosine and acts as a sort of chemical vehicle inside which iodination of tyrosine proceeds (Fig 12) In this way tyrosine is converted into monoiodotyrosine and diiodotyrosine Subsequently two molecules of diiodotyrosine couple with the loss of one side chain to form a molecule of tetraiodothyronine (thyroxine) —

Absorption Iodine is absorbed from the alimentary canal and to a lesser extent from the skin lungs and mucous membranes By and large it is absorbed in proportion to the solubility of the iodine compounds concerned

Storage Iodine reaches the blood stream as inorganic iodide and from the blood the thyroid removes as much iodide as it requires The thyroid gland is the only organ in the body in which appreciable quantities of iodine are stored

Excretion Inorganic iodide circulates passively in the blood and that which is not required by the thyroid is excreted by the kidneys sweat bile and saliva Competition between the thyroid and the kidneys is set up soon after iodide enters the blood and urinary levels are largely proportional to the intake There is no evidence that a renal threshold for iodide exists

Blood Iodine Normally blood cells contain only minute traces of iodine in the plasma iodine exists in two main forms The fraction which is attached to protein (protein bound iodine) is closely associated with variations in thyroid activity and is thought to represent the iodine of the circulating thyroid hormone The inorganic iodide which remains in the supernatent fluid after the serum proteins are precipitated is much more variable but usually much smaller in amount than the protein bound iodine it is a reflection of the amount of iodide absorbed from the alimentary tract The total body iodine content is about 50 mgm of which 10 to 15 mgm are to be found in the thyroid gland Protein bound iodine is normally present in blood in concentrations of between 3 and 8 μgm per 100 ml while inorganic iodide is present in levels of about 1 μgm per 100 ml

Requirements The body appears to use about 0 33 mgm of thyroxine daily and would be expected to require about 0 2 mgm of iodine to synthesise this amount of thyroxine[6] Actually the body manages on about 15 μgm of iodine daily[7], because it shows great economy in handling the element and retains most of that liberated from the metabolism of thyroxine

Synthesis of Thyroid Hormone

The first step in the production of thyroid hormone consists of the accumulation of iodine within the gland This in itself is a two fold process involving firstly the trapping of iodide and secondly the oxidation of iodide to free iodine In other words the thyroid concentrates large amounts of circulating inorganic iodide in the

Storage and Release of Thyroid Hormone

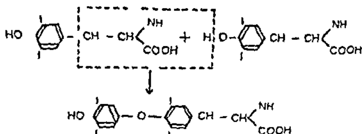
A feature of normal thyroid function is the capacity of the gland to provide the required amount of hormone at the right time This is achieved by enzymatic building up of thyroglobulin and its enzymatic splitting down into more soluble fractions such as thyroxine In this form the hormone can diffuse from or through the thyroid cell and enter the blood where it is quickly attached to protein molecules not by chemical combination but by adsorption That is to say the thyroid hormone can be stored in the colloid and released as required—passing through the thyroid cell on its way to the blood stream—or under more pressing circumstances it can be secreted directly from the cell into the circulation[11] (Fig 12)

Salter has suggested that the thyroid cell membrane is such that on its follicular aspect it can allow large molecules to pass freely whereas on its external aspect it will allow only smaller molecules to pass As a result thyroglobulin can only move towards the follicle and in so doing makes room for the synthesis of more protein In leaving the cell to enter the follicle thyroglobulin takes with it certain proteolytic enzymes which are capable of freeing the hormones from the larger molecule thus allowing them to pass through the thyroid cell into the circulation

Action of Thyroid Hormone

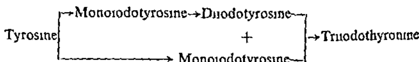
Our knowledge of the action of thyroid hormone is derived from the study of patients and experimental animals deprived of thyroid tissue on the one hand or subjected to an excess of the hormone on the other Present day conceptions of thyroid function do not permit of a detailed description of the integration of its activity within the physiology of the body as a whole For example certain disturbances of carbohydrate metabolism are seen in the experimental animals and patients mentioned above but the exact role played by the thyroid gland in carbohydrate metabolism under physiological conditions is not entirely clear The actions of the hormone are discussed under various headings for convenience but no doubt it will turn out that these manifold functions are really expressions of a few fundamental activities on the part of the hormone

1 *Calorigenic Activity* The thyroid hormone directly increases the rate of oxidation within the cells of the tissues In this respect



In addition to thyroxine, the thyroglobulin molecule contains triiodothyronine which may result from the conjugation of mono and diiodotyrosines or from the loss of one iodine atom from thyroxine. This suggests two possible methods for the synthesis of these iodinated amino acids which may be indicated thus —

Tyrosine → Monoiodotyrosine → Diiodotyrosine → Thyroxine and



The synthesis of thyroxine requires the intact thyroid cell[9]. It appears that the thyroid cell continuously produces a large protein molecule and stores this within the follicles as colloid. If the gland receives iodine it iodinates the tyrosine present within the protein molecule. This iodination can apparently take place within the cell or within the follicle.

The apparent simplicity of the synthesis of thyroxine by the thyroid gland has prompted certain workers to attempt this synthesis outside the thyroid gland. It has been shown that thyroxine can be prepared by incubating iodine (not iodide) with a number of proteins containing tyrosine; this reaction does not appear to be controlled by enzymes. Furthermore there is some evidence to show that both triiodothyronine and thyroxine can be formed in the rat outside the thyroid gland[10]. The ease with which thyroxine is formed under these conditions may well cause one to wonder why the body requires a thyroid gland. There are three answers to this question. In the first place the thyroid is capable of converting iodide to iodine. Secondly the gland concentrates and stores both the hormone and the raw materials from which it is formed in such a way that thyroxine can be released as required by the tissues. Thirdly the thyroid is capable of synthesising the hormone much more readily than any other tissue and can do so under conditions which are subject to control.

Storage and Release of Thyroid Hormone

A feature of normal thyroid function is the capacity of the gland to provide the required amount of hormone at the right time. This is achieved by enzymatic building up of thyroglobulin and its enzymatic splitting down into more soluble fractions such as thyroxine. In this form, the hormone can diffuse from or through the thyroid cell and enter the blood where it is quickly attached to protein molecules not by chemical combination but by adsorption. That is to say, the thyroid hormone can be stored in the colloid and released as required—passing through the thyroid cell on its way to the blood stream—or under more pressing circumstances it can be secreted directly from the cell into the circulation[11] (Fig 12)

Salter has suggested that the thyroid cell membrane is such that on its follicular aspect it can allow large molecules to pass freely whereas on its external aspect it will allow only smaller molecules to pass. As a result thyroglobulin can only move towards the follicle and in so doing makes room for the synthesis of more protein. In leaving the cell to enter the follicle thyroglobulin takes with it certain proteolytic enzymes which are capable of freeing the hormones from the larger molecule thus allowing them to pass through the thyroid cell into the circulation

Action of Thyroid Hormone

Our knowledge of the action of thyroid hormone is derived from the study of patients and experimental animals deprived of thyroid tissue on the one hand or subjected to an excess of the hormone on the other. Present day conceptions of thyroid function do not permit of a detailed description of the integration of its activity within the physiology of the body as a whole. For example certain disturbances of carbohydrate metabolism are seen in the experimental animals and patients mentioned above but the exact role played by the thyroid gland in carbohydrate metabolism under physiological conditions is not entirely clear. The actions of the hormone are discussed under various headings for convenience but no doubt it will turn out that these manifold functions are really expressions of a few fundamental activities on the part of the hormone

1 *Calorigenic Activity* The thyroid hormone directly increases the rate of oxidation within the cells of the tissues. In this respect

it probably acts as a catalyst In addition through variations in its activity it enables the body to meet changing physiological requirements The thyroid gland is responsible for about 40 per cent of the total metabolic activity of the body In other words the gland maintains the metabolic activity of the tissues at a higher level than they would otherwise achieve This action of the thyroid gland can be measured by studies of the basal metabolic rate (page 71)

2 *Growth Maturation and Differentiation of Tissues* In man and in vertebrate animals it is impossible to attain adult form and dimensions in the absence of thyroid hormone This action of the hormone is essential for the metamorphosis of amphibia The influence of thyroid hormone upon the rate of growth of cells has been shown by the stimulating effect which it exerts upon certain cells growing in tissue cultures It would seem that this influence is particularly marked in the case of skin nails hair, teeth and bone

3 *Electrolyte and Protein Metabolism* One of the conspicuous features of lack of thyroid hormone is the deposition of a mucoprotein substance between the body cells This protein is thought to be the same as that which constitutes the ground substance or cement normally found between the cells of the body Together with this protein there occurs an extracellular retention of salt and water a considerable reduction in plasma volume and an increased concentration of serum and spinal fluid proteins When thyroid hormone is administered under these conditions it produces a considerable diuresis during which fluid containing appreciable quantities of sodium is excreted However in normal individuals thyroid also has a diuretic action but under these circumstances the fluid excreted is especially rich in potassium suggesting that it is intracellular in origin

Byrom[1] has extended these and other observations to provide an attractive theory for the basic action of thyroid hormone He suggests that the fundamental activity of the gland is directed towards the removal of the mucoprotein in which the foetal tissues are embedded This has the effect of freeing the cell surface for those metabolic exchanges which will enable growth and division of cells to proceed more rapidly In Byrom s view the foetal tissues are choked by mucoprotein which prevents the rapid exchanges of ions and water so necessary for cell growth and division this is overcome by the removal of the extracellular protein and so cell multiplication proceeds at an ever increasing

rate Such a concept provides a satisfactory explanation of thyroid activity but as yet it remains speculative

4 *Carbohydrate Metabolism* Our knowledge of the part played by thyroid hormone in carbohydrate metabolism consists of a number of disjointed facts Thyroid hormone accelerates the rate of absorption of sugar from the alimentary canal It further depletes the liver of glycogen (perhaps indirectly as the result of increased oxidation of sugar by the body tissues) The hormone also delays the deposition of glycogen in the liver during the process of refeeding after starvation its basic action is thought to be in the liver where it increases the mobilization of glycogen

In addition the hormone affects carbohydrate metabolism indirectly as a result of its stimulating effect upon body metabolism as a whole Moreover it is capable of promoting the reabsorption of glucose by the renal tubules i e of raising the renal threshold for glucose

5 *Lipoid Metabolism* Hypothyroidism is usually associated with a rise in serum cholesterol and conversely (though with less regularity) hyperthyroidism with a fall It has been suggested that under these conditions the total body cholesterol is unaltered the changes observed being the result of movement of cholesterol to and from the plasma

6 *Bone* Thyroid hormone is essential to the normal development of bone Epiphysial fusion is delayed and the epiphyses themselves show characteristic radiological changes when the hormone is not present in normal quantities before puberty

7 *Nervous System* An excess of thyroid hormone increases the irritability of the nervous system a lack produces the opposite effect These observations suggest that the thyroid hormone is one factor responsible for the equitable activity of a healthy nervou system The higher cerebral centres reflect changes in thyroid function—heightened emotional activity failure of concentration and flight of ideas appear in the presence of overactivity By contrast a slowness and confusion of thought together with a lack of emotion and loss of memory are characteristic of underactivity of the gland The autonomic nervous system reacts to an excess of thyroid hormone by producing increased vasomotor activity (flushing and peripheral vasodilatation) increased peristalsis and excessive activity of sweat glands

8 *Muscular System* In addition to those changes which result from the action of thyroid hormone upon the nerves supplying

muscles, excess of the hormone causes destruction of muscular tissue with an increased excretion of creatine

9 *Cardiovascular System* Thyroid hormone in addition to acting upon the heart indirectly by way of the nervous system and the general metabolic stimulation already mentioned also exerts certain direct effects The isolated perfused heart shows an increase in rate and stroke volume in response to the addition of thyroxine to the perfusing fluid[13] The result of these various effects in the intact animal is to produce tachycardia peripheral vasodilatation increased stroke volume and an increase in pulse pressure There is also said to be a shift in the oxygen dissociation curve brought about by thyroxine which facilitates the delivery of oxygen to the tissues in answer to the demands resulting from increased metabolic activity[14]

10 *Tissue Healing* The healing and regeneration of injured tissue is delayed by lack of thyroid hormone What part the thyroid gland plays in wound healing under normal conditions cannot be stated

11 *Blood Cells* Thyroid hormone stimulates bone marrow and is necessary in man for normal quantitative and qualitative red cell production It is also thought that thyroid hormone may play a part in the production of platelets

12 *Endocrine Glands* The thyroid gland functions in harmony with its fellow endocrine glands As yet little is known of the details of these relationships beyond a number of isolated facts which have emerged from experimental studies The best documented of these facts are given below but it is not possible to amplify them in such a way as to give full meaning to such observations The relationship between the thyroid and the adenohypophysis is the most important of these integrating activities and will be discussed in dealing with the control of thyroid activity

(i) *Thyroid Gland* Thyroid hormone might be expected to exert its widespread stimulating effect upon the metabolism of the thyroid cells However, there is some evidence to show that the hormone exerts a direct inhibitory effect upon the cells of the gland In addition it displays an indirect inhibition brought about by depression of anterior pituitary secretion of thyroid stimulating hormone

(ii) *Adrenal Medulla* Adrenaline appears to depress thyroid activity as shown by a fall in the uptake of iodine by the thyroid gland after adrenaline has been injected into an adrenalectomised animal kept alive by means of adrenocortical extracts

(iii) *Adrenal Cortex* Thyroid hormone when administered in excess causes cortical hypertrophy and hastens the hypertrophy of the remaining adrenal when its fellow has been removed By contrast, removal of the thyroid gland causes atrophy of the cortex[15]

(iv) *Gonads* Diseases of the thyroid gland are so regularly associated with changes in the function of the gonads that a specific relationship between the two has long been suspected So far the extent and nature of such a functional association is not clear Present day studies are directed towards the anterior pituitary gland which appears to take part in a triangular relationship with the thyroid and the gonads

(v) *Posterior Pituitary* Disease of or damage to the posterior pituitary gland gives rise to a condition involving gross polyuria (page 177) this polyuria only occurs in the presence of normal thyroid function No entirely satisfactory explanation can be given to account for this observation

13 *Vitamin Metabolism* Thyroid hormone by stimulating the overall metabolism of the body tissues increases the demand for vitamins in general and if this demand be not satisfied in the presence of an excess of the hormone relative vitamin deficiency may result In addition however a voluminous literature has appeared in which the question of a more specific interaction between thyroid hormone and vitamin metabolism is suggested Vitamin A metabolism seems to involve some specific relationship with thyroid hormone the nature of which is not clear Excess of vitamin A appears to depress thyroid function on the other hand in the absence of thyroid hormone it appears that some species are incapable of converting carotene to vitamin A and an excess of carotene in the body may result

Metabolism of Thyroid Hormone

Although much has been written about the effects produced by thyroid hormone little is known of just how such effects are brought about or of the fate of the hormone after it has acted upon the tissues of the body One of the most striking features about the action of thyroxine when it is used to replace the natural hormone of thyroidectomised animals is the latent interval which elapses between a single injection of the hormone and the first detectable change which results This latent period has been a source of interest to experimental physiologists who have specu

lated that it might be occupied by the chemical transformation of thyroxine in the tissues into one or more active derivatives

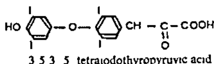
Recent studies of the acetic acid derivatives called TETRAC and TRIAC have suggested that these substances may represent the active compounds into which the circulating hormones are converted by the tissues[24 25] Some workers have even gone so far as to suggest that different tissues contain enzymes capable of converting the circulating hormone into a number of active derivatives each of which possesses a particular function not shown (or shown in lesser degree) by the others In this way it has been shown that triiodothyroacetic acid has little effect upon the oxygen consumption of tissues but exerts a potent effect upon metamorphosis Similarly propionic acid derivatives promote metamorphosis without stimulating tissue metabolism[25] Tetraiodothyroacetic acid on the other hand stimulates tissue metabolism but exerts little effect upon metamorphosis These studies have so far given most encouraging results but further elaboration of the detailed actions of the various derivatives must await subsequent developments

In the last analysis the mechanism of action of the thyroid hormones is not known However, it has been shown that thyroxine uncouples a proportion of the phosphorylations which occur during the oxidation of β hydroxybutyrate Although this effect of thyroxine upon oxidative phosphorylation is important in that it suggests that the hormone may act by affecting enzyme systems in the body the discovery was made in vitro and its physiological significance is at present obscure

This uncoupling of phosphorylation is associated with increased cellular respiration Among the best studied actions of thyroxine is its effect upon the enzymatic oxidation of ascorbic acid Thyroxine inhibits the copper catalysed oxidation by removing the metal as an insoluble complex composed of one mole of cupric chloride and three moles of thyroxine Other enzyme systems are inhibited by thyroxine and this inhibition has been shown to result from the removal of magnesium by means of the formation of a complex with the hormone [7a]

Among the means available to the body for disposing of thyroid hormones excretion and inactivation by the liver are amongst the most important It has been shown that the liver not only possesses enzymes capable of deiodinating thyroxine but that it can convert this compound into TETRAC and may in this way influence the utilization of thyroid hormone by the tissues Moreover the liver excretes thyroxine in the bile in the form of glucuronic acid com

pounds (i e the process of conjugation page xiii) Again certain tissues can oxidise thyroxine without deiodination in such a way as to deaminate the compound giving iodopyruvic acid Pyruvic acid derivatives are found in the bile, e g —



3 5 3 5 tetraiodothyropyruvic acid

Finally dehalogenation which liberates the iodine from the hormone occurs in certain tissues [5]

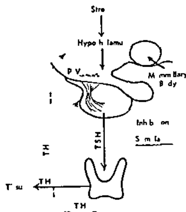


Fig 13 The control of the thyroid gland
T H Thyroid Hormone P V Portal Vessels

Control of the Thyroid Gland

The thyroid gland is phylogenetically an old structure and one which has later come under the controlling influence of the anterior pituitary gland It is generally agreed that the anterior pituitary controls the thyroid by the secretion of a trophic hormone —the thyrotrophic or thyroid stimulating hormone (TSH) (Fig 13) Relatively pure preparations of TSH have recently been made available for experimental studies

1 *ACTION OF TSH*[11]

Thyrotrophic hormone has two important actions —

(1) *Thyroid Stimulation* and (2) *Systemic Actions* Thyroxine antagonises both of these effects while iodine mitigates only the first action

1) *Thyroid Stimulation* The stimulating effect of TSH upon the thyroid is revealed by histological and anatomical changes in the gland as well as by certain functional changes

A) *Histological and Anatomical Changes*

a) Increase in the number of intracellular droplets within the thyroid cells

b) Increase in the height of the thyroid cells and when this has reached a maximum increase in the number of these cells

c) Increase in the vascularity of the gland

d) Increase in the weight of the thyroid gland

B) *Functional Changes*

a) Increased uptake of iodide by the thyroid

b) Increased production of thyroid hormone

c) Increased discharge of hormone into the circulation this may represent the fundamental action of TSH

These changes are associated with acceleration of the rate of metamorphosis of amphibia and in excess with the signs of hyper thyroidism The release of thyroid hormone from the thyro globulin molecule probably results from the action of proteolytic enzymes such an enzyme system is known to exist within the colloid These enzymes transform thyroglobulin into smaller molecules which can diffuse through the thyroid cell and so enter the circulation the exact composition and relative proportions of these smaller molecules (i e the thyroid hormones) remain uncertain but as mentioned above thyroxine is the most important

2) *Systemic Actions* [8]

a) *Orbit* TSH causes an increase in the water content of orbital structures and an increase in orbital fat Extraocular muscles become swollen and hypotonic and show fragmentation and infiltration with polymorphonuclear cells

b) *Miscellaneous* Certain effects have been described following the experimental use of TSH but their significance under normal conditions is doubtful The hormone produces water retention an increase in plasma fat and blood acetone together with a rise in the number of circulating polymorphonuclear cells the polymor phonuclear cells show fat droplets in the cytoplasm Finally TSH causes skeletal muscles to lose their striations and to show minute intracellular fat droplets

These actions of TSH have been studied in experimental ani

mals and it should be realised that the conditions of the experiments concerned are highly unphysiological The truth is not as clear cut as these statements might suggest Some observers for example attribute the action of TSH upon the orbital structures to a hor mone called the exophthalmos producing substance (EPS) which produces minimal thyroid stimulation but is closely associated with TSH in extracts of the adenohypophysis [74]

The stimulating effect of TSH upon the thyroid gland is diminished by iodide and by thyroxine It is probable that the mechanism in each case is different Iodide is converted to iodine by the thyroid and free iodine is capable of inactivating TSH by oxidation Thyroxine depresses TSH activity by causing a fall in the rate of its production or its release from the anterior pituitary This seems to be the mechanism of mutual stimulation and inhibition which controls the activity of the gland under physiological conditions There is a basic autonomous output of thyroid hor mone which is increased by the action of TSH the secretion of TSH is kept in check by the depressing effect of thyroxine upon the production of the trophic hormone by the pituitary and thy roxine also depresses the production of hormone by the thyroid gland itself In this way a self regulating mechanism is set up In addition the possibility remains that inactivation of TSH may result under normal circumstances from its utilization by the thyroid gland (Fig 13)

Other factors which are known to influence thyroid activity include iodine deficiency changes in environmental temperature and certain chemical compounds called antithyroid substances

2 *IODINE*

In the absence of adequate iodine the thyroid cannot fulfil the demand of the body for thyroid hormone Excess of iodine on the other hand usually does not affect the thyroid activity of a normal person unless a very high concentration is reached However the function of an overactive thyroid gland can be depressed at least temporarily by the administration of iodine The mechanism of this action is not fully understood it may involve suppression of iodide uptake by the gland or it may inter fere with the action of thyroid stimulating hormone It has been shown that following the administration of iodine to patients suffering from excessive thyroid activity there occurs a rise in the level of circulating TSH [1] Finally it is clear that iodine does not act peripherally in the tissues by interfering with the action of thyroxine

3 *TEMPERATURE*

The temperature of the environment may affect thyroid function by way of the hypothalamus It is probable as Notila[19] has suggested, that the basic regulation of the thyroid gland is humoral and is brought about by variations in the concentration of thyroxine in the blood However under certain circumstances this may be modified by the hypothalamus The stimuli affecting the hypothalamus under such circumstances include changes of temperature and exposure of the body to stresses of various kinds (page 44)

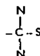
4 *ANTITHYROID SUBSTANCES*

This term is applied to a group of substances which share the property of preventing in one way or another the synthesis of thyroid hormone The term goitrogenic is also applied to these substances and the goitre so produced results from the stimulating effect of TSH Failure of the thyroid to produce its hormone causes a fall in the blood level of thyroxine which in turn stimulates the anterior pituitary to produce TSH and this brings about hypertrophy of the thyroid gland leading eventually to the appearance of a goitre Antithyroid substances in general have the common property of causing hyperplasia of the thyroid gland together with a fall in the production of thyroid hormone

The thyroid hormone is synthesised as the result of a series of chemical reactions consequently antithyroid drugs can produce their effect at different levels

(i) *Thiocyanate* The antithyroid action of thiocyanate ion differs from that of other such agents in that it is readily overcome by the concomitant administration of iodine and may be due at least partly to its ability to inhibit the uptake of iodide by the gland Thiocyanate is widely distributed in nature and may be a factor in the occurrence of endemic goitre in districts where the iodine content of soil and water is abundant

(ii) *Thiocarbonamides* A number of substances including thiourea and thiouracil containing a thiocarbonamide grouping



possess antithyroid properties These compounds do not affect the capacity of the gland to accumulate iodine but may interfere with the iodination of tyrosine because they react with the element much more rapidly than does tyrosine

(iii) *Aminobenzenes* A number of compounds which possess

an aminobenzene grouping ⌬NH are capable of exerting an antithyroid effect It has been suggested that this group of substances which includes the sulphonamides may act by interfering with the conversion of diiodotyrosine to thyroxine but the data at present available are not conclusive

Tests of Thyroid Function

Clinical and physiological laboratories now possess a number of tests each of which is capable of measuring some aspect of thyroid function It is well to remember that each group of tests measures some different aspect of thyroid activity and that each throws some light on thyroid function from a different angle they are complementary rather than mutually exclusive

The Basal Metabolic Rate[4] This term may be defined as the heat production per square metre of body surface in unit time by a subject who is at complete physical and mental rest and in the postabsorptive state The postabsorptive state involves a period of 12 to 14 hours fasting after which it is assumed that the digestive processes are at a standstill The subject rests for 30 minutes before the test is performed Although the metabolic rate is defined in terms of heat production for clinical purposes it is usual to measure the rate at which oxygen is used this gives an indirect measure of heat production The usual apparatus is some modification of the Benedict Roth machine in which the patient rebreathes pure oxygen the carbon dioxide produced being removed by passing the expired air through soda lime

The surface area of the body is calculated from the height and weight of the subject In this way it is possible to calculate the volume of oxygen used per square metre of body surface area per minute The observed oxygen consumption is compared with that of a normal person of the same age and sex and the result is expressed as a percentage of above (+) or below (−) normal Normal people usually fall within the range of +10 to −10 per cent but the exact range will differ from laboratory to laboratory Although a number of physiological and pathological factors affect the BMR, if these be taken into consideration the test gives some measure of the activity of the thyroid gland at the time of testing

Serum Cholesterol The normal range of serum cholesterol is said to lie between 150 and 240 mgm per 100 ml although each group of workers needs to establish its own range of normal

3 *TEMPERATURE*

The temperature of the environment may affect thyroid function by way of the hypothalamus It is probable as Notila[19] has suggested that the basic regulation of the thyroid gland is humoral and is brought about by variations in the concentration of thyroxine in the blood However under certain circumstances this may be modified by the hypothalamus The stimuli affecting the hypothalamus under such circumstances include changes of temperature and exposure of the body to stresses of various kinds (page 44)

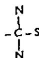
4 *ANTITHYROID SUBSTANCES*

This term is applied to a group of substances which share the property of preventing in one way or another the synthesis of thyroid hormone The term goitrogenic is also applied to these substances and the goitre so produced results from the stimulating effect of TSH Failure of the thyroid to produce its hormone causes a fall in the blood level of thyroxine which in turn stimulates the anterior pituitary to produce TSH and this brings about hypertrophy of the thyroid gland leading eventually to the appearance of a goitre Antithyroid substances in general have the common property of causing hyperplasia of the thyroid gland together with a fall in the production of thyroid hormone

The thyroid hormone is synthesised as the result of a series of chemical reactions consequently antithyroid drugs can produce their effect at different levels

(i) *Thiocyanate* The antithyroid action of thiocyanate ion differs from that of other such agents in that it is readily overcome by the concomitant administration of iodine and may be due at least partly to its ability to inhibit the uptake of iodide by the gland Thiocyanate is widely distributed in nature and may be a factor in the occurrence of endemic goitre in districts where the iodine content of soil and water is abundant

(ii) *Thiocarbonamides* A number of substances including thiourea and thiouracil containing a thiocarbonamide grouping



possess antithyroid properties These compounds do not affect the capacity of the gland to accumulate iodine but may interfere with the iodination of tyrosine because they react with the element much more rapidly than does tyrosine

(iii) *Aminobenzenes* A number of compounds which possess

(exophthalmos) is seen and is thought to result from excess TSH This observation has given rise to the suggestion that cases of hyperthyroidism showing exophthalmos may result from abnormal function of the anterior pituitary gland Hyperthyroidism may occur de novo or it may follow the development of a goitre

HYPOTHYROIDISM Hypothyroidism is the antithesis of hyperthyroidism and individuals so affected become sluggish in their mental and physical behaviour The pulse is slow the skin dry and deposits of mucoprotein occur throughout the body

REFERENCES

As in other chapters of this book only a selection of the more important references is given "The Thyroid and Its Diseases by J H Means (Lippincott) apart from its excellent chapters which deal with thyroid physiology gives references to every aspect of the subject "Diseases of the Endocrine Glands" by L J Soffer (Lea & Febiger Philadelphia 1956) also contains an exhaustive survey of the literature

1 Hartley J N J, Surg Gynaec & Obst 35 543 1922
2 Gotesch E. Bull J Hop Hosp 27 129 1916
3 Uhlenhuth E. Quart J Micr Sci 76 615 1934
4 Porter K R et al J Exper Med 81 233 1945
5 Harrington C R. Lancet 1 1199 1261 1935
6 Plummer H S and Boothby W M Am J Phys 55 295 1921
7 Eggenberger H Sweite Internet Kropfkonferenz, Bern (1933) Verhandlungsbericht 454 1935
7a Modern Trends in Endocrinology Butterworth 1958
8 Keating F R. et al J Clin Invest 26 1138 1947
9 Morton M E & Chaikoff I L J Biol Chem 147 1 1943
10 Morton M E et al J Biol Chem 147 757 1943
11 Means J H The Thyroid and Its Diseases p 33 Lippincott 1948
12 Byrom F B Clin Sc 1 273 1934
13 Leblond C P & Hoff H E Am J Physiol 141 32 1944
14 Zondek H The Diseases of the Endocrine Glands Wood Baltimore 1935
15 Ingle D J & Higgins G M Endocrinology 23 419 1938
16 Kendall E C Endocrinology 24 798 1939
17 Haffman F & Hoffman E J Publications del Institute de Fiziologie Universided de Chile 1944
18 Williams R H Endocrinology p 18 Saunders Philadelphia 1950
19 Uotila U U Endocrinology 26 129 1940
20 Du Bois E F Basal Metabolism in Health & Disease Philadelphia Lea. 1936
21 Soffer L J Diseases of the Endocrine Glands p 800 Lea & Febiger Philadelphia 1956
22 De Robertis E Anat Rec 80 219 1948
23 Rawson R W J Clin Endocrinol 16 1405 1956

Underactivity of the thyroid gland is associated with a high serum cholesterol, while the converse is true but with less regularity

Serum Protein Bound Iodine Normal individuals show between 3 and 8 μgm of protein bound iodine per 100 ml The concentration of protein bound iodine in the blood can be estimated chemically and this investigation provides a measure of the concentration of thyroid hormone in the blood but it is difficult to perform accurately

Radioactive Iodine[21] An ever increasing number of tests involving the use of radioactive iodine have been devised to measure the avidity of the thyroid gland for iodine I^{131} with a half life of 8 days is the isotope almost universally employed in these tests The avidity of the gland for iodide is a measure of the state of its functional activity the more iodide it takes up the more active the gland

DISEASES OF THE THYROID GLAND

Some aspects of thyroid function are aptly illustrated by diseases of the gland With this in mind brief reference is made to the more important diseases of the gland

GOITRE The word goitre is derived from guttur meaning throat and is used in clinical medicine to refer to any enlargement of the thyroid gland which is not due to neoplasm or to inflammatory disease *Goitre is endemic in certain regions of the world* and its distribution is closely associated with areas in which the soil and water are deficient in iodine This association is of such an order that it is generally held that iodine deficiency plays some part in the development of endemic goitre In addition however certain substances occur in the diet which are called antithyroid or goitrogenic and these may produce endemic goitre It has recently been shown for example that some antithyroid factor is present in milk in parts of Tasmania Sporadic goitre on the other hand is world wide in its distribution and may occur in families Some families are known in which certain members inherit a *defect which* interferes with the synthesis of thyroid hormone with the result that an excess of TSH following the low rate of secretion of thyroid hormone produces a goitre

HYPERTHYROIDISM Patients so affected demonstrate all the signs which would be expected from the known actions of the hormone Loss of weight nervousness and irritability rapid pulse profuse sweating and tremor of the fingers are among the signs of hyperthyroidism Not infrequently protrusion of the eyeballs

(exophthalmos) is seen and is thought to result from excess TSH This observation has given rise to the suggestion that cases of hyperthyroidism showing exophthalmos may result from abnormal function of the anterior pituitary gland Hyperthyroidism may occur de novo or it may follow the development of a goitre

HYPOTHYROIDISM Hypothyroidism is the antithesis of hyperthyroidism and individuals so affected become sluggish in their mental and physical behaviour The pulse is slow the skin dry and deposits of mucoprotein occur throughout the body

REFERENCES

As in other chapters of this book only a selection of the more important references is given "The Thyroid and Its Diseases by J H Means (Lippincott) apart from its excellent chapters which deal with thyroid physiology gives references to every aspect of the subject. "Diseases of the Endocrine Glands by L. J Soffer (Lea & Febiger Philadelphia 1956) also contains an exhaustive survey of the literature

1 Hartley J N J Surg Gynaec & Obst 35 543 1922
2 Gotesch E Bull J Hop Hosp 27 129 1916
3 Uhlenhuth E Quart J Micr Sci 76 615 1934
4 Porter K R et al J Exper Med 81 233 1945
5 Harrington C R Lancet 1 1199 1261 1935
6 Plummer H S and Boothby W M Am J Phys 55 295 1921
7 Eggenberger H Sweite Internet Kropfkonferenz Bern (1933) Verhandlungsbericht 454 1935
7a Modern Trends in Endocrinology Butterworth 1958
8 Keating F R et al J Clin Invest 26 1138 1947
9 Morton M E & Chaikoff I L J Biol Chem 147 1 1943
10 Morton M E et al J Biol Chem 147 757 1943
11 Means J H The Thyroid and Its Diseases p 33 Lippincott 1948
12 Byrom F B Clin Sc 1 273 1934
13 Leblond C P & Hoff H E Am J Physiol 141 32 1944
14 Zondek, H The Diseases of the Endocrine Glands Wood Baltimore 1935
15 Ingle D J & Higgins G M Endocrinology 23 419 1938
16 Kendall E C Endocrinology 24 798 1939
17 Haffman F & Hoffman E J Publications del Institute de Fiziologie Universided de Chile 1944
18 Williams R H Endocrinology p 18 Saunders Philadelphia 1950
19 Uotila U U Endocrinology 26 129 1940
20 Du Bois E F Basal Metabolism in Health & Disease Philadelphia Lea. 1936
21 Soffer L J Diseases of the Endocrine Glands p 800 Lea & Febiger Philadelphia 1956
22 De Robertis E Anat Rec 80 219 1948
23 Rawson R W J Clin Endocrinol 16 1405 1956

24 Lerman J & Pitt Rivers R J Clin Endocrinol 16 1470 1956
25 Ciba Colloquia on Endocrinology volume 10 1957 gives a detailed account of recent advances in the study of thyroid physiology together with references to the literature on this subject

CHAPTER IV

THE TESTIS

Introduction

It is the responsibility of the testis to equip the adult male for his role in the function of reproduction This is achieved by means of two distinct but related mechanisms In the first place the internal secretion of the testis brings about the appearance of secondary sexual characteristics which distinguish the adult male and also prepares the accessory genital organs so that they may successfully transport the germinal product of the testis Secondly the testis carries out the genetic and morphological activities which produce the male sex cell

Embryology

The genital organs of vertebrate animals share a common origin with the urinary organs Anlagen for the genital organs of both sexes exist in the early embryo and sexual differentiation consists in an orderly elaboration of homologous organs together with degeneration of heterologous counterparts a few of the latter remain in the adult as vestigial remnants The urinary system produces in succession three different types of excretory organs situated one behind the other only the third of these structures survives as a functional entity in man In this way the foetus gives a classical illustration of the doctrine of phylogeny since it produces two excretory organs which function only in lower animals

The first excretory organ is the pronephros which is functionless in man it leaves behind only its duct (the Wolffian duct) which is used by the mesonephros or second excretory organ (Fig 14) The mesonephros begins as a series of tubules but eventually gives rise to an important part of the male genital tract its maximal elaboration at the seventh week coincides with the differentiation of the gonad into a testis or an ovary As some of the mesonephric tubules and their adopted duct become incorporated into the genital organs the cranial portion undergoes atrophy and the whole structure is reinforced by the formation of new tubules caudally in this way the mesonephros is relegated to a more caudal posi

tion By the end of the fifth month the degeneration of redundant mesonephric tubules is complete At this stage the excretory function is assumed by the third or definitive excretory organ the metanephros (Fig 14)

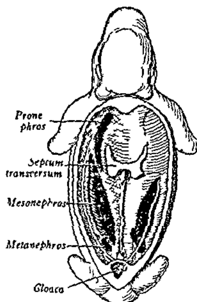


Fig 14 The three excretory organs of the embryo seen in a diagrammatic dissection of the trunk from the ventral surface The left side of the embryo shows a later stage than the right (*Arey*)

The sex of an individual is the outcome of two distinct pro cesses namely sex determination and sex differentiation Sex determination is a genetic phenomenon and results in the genetic sex or sex genotype of the embryo Sex differentiation on the other hand refers to those changes which determine the course of embryonic development of the gonads the genital ducts and the external genital organs

SEX DETERMINATION The somatic sex of an infant is the result of two developmental processes namely the direction of differentiation of the gonads and the development of the genital tracts It is generally held that these two processes are predeter mined at the moment of fertilization by the particular chromo somal pattern of the zygote, that is to say sex determination is believed to govern the direction of sex differentiation Of the 48 chromosomes present in all somatic and immature germ cells of man two are partly responsible for the determination of sex and

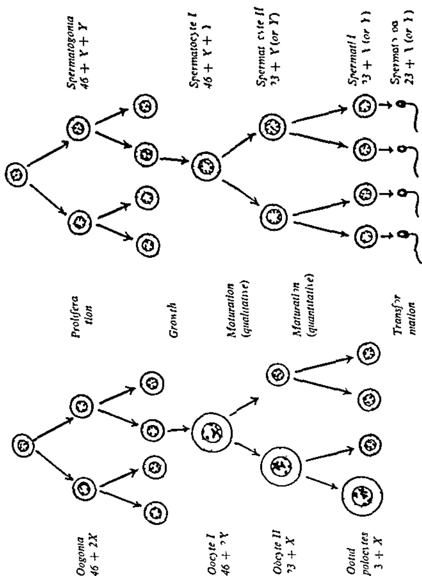


Fig 15 Diagram illustrating oogenesis and spermatogenesis The stages of proliferation growth and maturation are shown The assortment of chromosomes is indicated at each stage (*Arey*)

tion By the end of the fifth month the degeneration of redundant mesonephric tubules is complete At this stage the excretory function is assumed by the third or definitive excretory organ the metanephros (Fig 14)

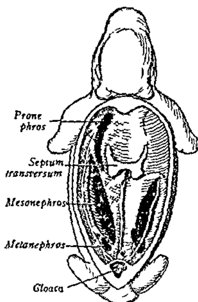


Fig 14 *The three excretory organs of the embryo seen in a diagrammatic dissection of the trunk from the ventral surface The left side of the embryo shows a later stage than the right (Arey)*

The sex of an individual is the outcome of two distinct processes, namely sex determination and sex differentiation Sex determination is a genetic phenomenon and results in the genetic sex or sex genotype of the embryo Sex differentiation on the other hand refers to those changes which determine the course of embryonic development of the gonads the genital ducts and the external genital organs

SEX DETERMINATION The somatic sex of an infant is the result of two developmental processes namely the direction of differentiation of the gonads and the development of the genital tracts It is generally held that these two processes are predetermined at the moment of fertilization by the particular chromosomal pattern of the zygote that is to say sex determination is believed to govern the direction of sex differentiation Of the 48 chromosomes present in all somatic and immature germ cells of man two are partly *responsible for the determination of sex and*

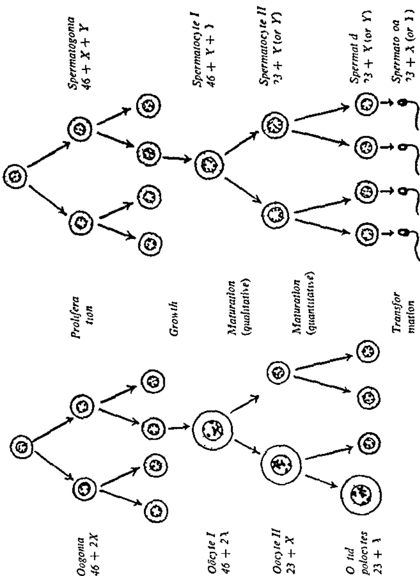


Fig 15 Diagram illustrating oogenesis and spermatogenesis The stages of proliferation growth and maturation are shown The assortment of chromosomes is indicated at each stage (*Arey*)

are designated X and Y, of these the X chromosome is the more active the Y chromosome being vestigial In females there are two X chromosomes in all somatic and immature germ cells while in males there occur one X and one Y

During the first of the specific processes of division (meiosis) which give rise to the male germ cell, the pair of sex chromosomes separate and one goes into each of the resulting secondary spermatocytes (Fig 15) This means that each of the two secondary spermatocytes resulting from the division of a primary spermatocyte bears a different sex chromosome one contains an X chromosome the other a Y In the female the corresponding division produces two cells each of which carries an X chromosome Therefore the sex of the zygote will be determined by the male germ cell A male cell bearing 23+X chromosomes will produce a zygote containing 46+2X—a female one bearing 23+Y will produce 46+X+Y—a male zygote (Fig 15)

Recent studies of sex determination have indicated that this process is more complex than was formerly thought[36] It has been suggested by a number of workers that genetic sex is the outcome of a balance between the influence of the sex chromosomes on the one hand and that of an undefined number of genes carried by autosomal chromosomes on the other Goldschmidt[36] for instance believes that the X chromosome carries genes which favour a female genetic sex while the Y chromosome bears no genes capable of influencing sex determination He further believes that autosomal chromosomes carry only male determining genes According to this concept genetic sex represents the result of competition between the female directing genes carried on the X chromosome and the male directing genes of the autosomes Two X chromosomes are quantitatively sufficient to overcome the influence of the autosomes and therefore determine female genetic sex one X chromosome and one neutral Y chromosome fail to do so and therefore permit the autosomes to determine male genetic sex

SEX DIFFERENTIATION Human sex differentiation involves a series of events which transform the embryo from a potentially bisexual organism equipped with gonadal and genital duct primordia capable of developing in a male or female direction to a foetus with the genital organs appropriate to one or other sex These events involve the gonads in the first instance the genital ducts are next affected and finally the urogenital sinus and the external genitalia differentiate in one direction or the other Normally the development of all the genital apparatus is consistent

with the genetic sex established in the zygote at the time of fertilization

Differentiation of the gonads During the fifth week of embryonic life a bilateral longitudinal ridge called the genital ridge develops medial and parallel to the mesonephros (Fig 3) The surface of this ridge is covered by a thickening of the coelomic epithelium which is called the germinal epithelium This epithelium contains the primordial germ cells which are generally believed to have migrated into the genital ridge from some distant site such as the endoderm of the yolk sac The germinal epithelium proliferates, causing cords of cells to penetrate the underlying mesoderm these extensions of the germinal epithelium are called the primary sex cords and towards them other cords are seen to grow from the adjacent mesonephros

During the seventh week the specific histological changes which indicate that the gonad is committed to a male or to a female role are to be seen *The testis differentiates before the ovary beginning* with organisation of the primary sex cords within the medulla of the gonad These cords form the anlage of the seminiferous tubules and subsequently join the cords from the mesonephros to form a continuous network of tubules Mesenchymal cells in the medulla of the gonad give rise to the cells of Leydig which are first seen during the eighth week These mesenchymal cells are thought to be derived from the mesonephros (Fig 16)

The ovary is only distinguishable at first because it fails to show evidence of the elaboration characteristic of the testis By the tenth week the cortex of the ovary is seen to be composed of nests of epithelial cells together with primordial follicles each of which contains an ovum The medulla of the ovary contains small tubules and scattered groups of hilar cells which represent the ovarian counterpart of the cells of Leydig The cortex of the ovary is derived from the germinal epithelium while the medulla comes from the mesonephros (Fig 16)

As in the case of genetic sex determination recent studies of the embryology of the gonads indicate that gonadal development results from competition between male and female elements[37] The indifferent gonad consists of two discrete components (i) a cortex which contains the germinal epithelium and (ii) a medulla which contains the primary sex cords At the time of gonadal differentiation the primordial germ cells migrate into the developing gonad In the absence of this invasion by germ cells the sterile gonad takes on a form which resembles that of a normal testis regardless of the genetic sex of the organism

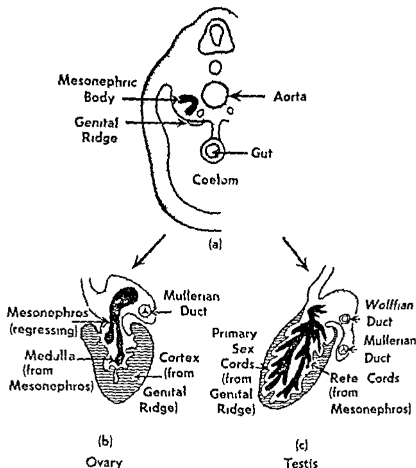


Fig 16 The development of the ovary and testis from the indifferent gonad The ovary shows cortical development together with medullary regression while the testis shows predominantly medullary development The lined areas represent the components derived from the germinal epithelium the contribution of the mesonephros is shown in black (*Lawson Wilkins*)

The cortex and the medulla are heterologous structures that is to say each can only develop along a defined path The cortex can only develop as an ovary the medulla only as a testis During differentiation *of the gonad these two elements compete for dominance* Under normal circumstances the dominant element conforms to the genetic sex of the organism and the recessive element undergoes regression

The primordial testis differentiates earlier than its ovarian counterpart and shows moreover, greater resistance to external

influences The primordial ovary begins to develop as a testis and only later alters in favour of ovarian development There is also good evidence to show that the primordial testis is able to secrete a substance capable of inhibiting ovarian (cortical) development

Differentiation of the genital ducts If the genital ducts are to develop in accordance with the sex of the gonads then either the Wolffian duct or the Mullerian duct must degenerate In the male foetus the Mullerian duct begins to degenerate during the twelfth week in the female the Wolffian duct begins to disappear one or two weeks later In the male the proximal portion of the Wolffian duct develops into the epididymis while the distal portion gives rise to the ductus deferens and to the prostate gland Only the extreme proximal extremity of the Mullerian duct remains and this appears in the form of functionless remnants

Development of the external genitalia The last event required to complete sex differentiation in the foetus is the development of the external genitalia By the twelfth week it is usually possible to distinguish a male foetus from a female by the appearance of the external genitalia In the male the phallus grows more rapidly and fusion of the labioscrotal folds occurs in the midline—beginning posteriorly and proceeding anteriorly to the base of the phallus In this way the urogenital sinus closes over with the formation of the perineal urethra Fusion continues along the ventral surface of the penis In the female fusion does not occur and the urogenital sinus gives rise to the vestibule

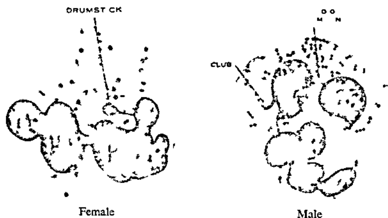


Fig 17 Two polymorphonuclear leucocytes The cell on the left shows the characteristic drumstick formation seen in female polymorphonuclear cells the cell on the right is a male polymorphonuclear and shows a small club

Jost[8] has shown that removal of the gonads from a foetus of either sex before the development of the genital ducts leads to the appearance of female internal and external genital organs regardless of the genetic sex of the foetus This observation is interpreted as meaning that the testis secretes some substance as yet unidentified which leads to the development of male genital organs and that in the absence of this substance(s) the genital ducts develop in a female direction

Recently it has become possible to identify the genetic sex of an individual by the appearance of the chromosomal material present in cells of the skin and oral mucosa or in polymorphonuclear cells (Fig 17) Such determinations of genetic sex have revealed that in man certain individuals show a discrepancy between somatic sex on the one hand and genetic sex on the other These observations emphasise the importance of distinguishing between sex determination and sex differentiation

Histology

The testis is composed of a series of tubules separated by interstitial tissue The tubules contain the germinal epithelium while the interstitial tissue contains the glandular cells which give rise to the internal secretion of the organ (Fig 18)

SEMINIFEROUS TUBULES The convoluted seminiferous tubule is composed of epithelial cells surrounded by a delicate homogeneous basement membrane The average diameter of adult tubules is between 150 and 250 μ Until puberty the tubular epithelium almost fills the lumen and cell differentiation has not begun The cells which line the tubules are of two varieties — the germinal epithelium and the cells of Sertoli

(i) *Germinal Epithelium* (Fig 18) The majority of the cells within the adult tubule comprise the germinal epithelium which consists of an orderly arrangement of some 4 to 8 layers of cells between the basement membrane and the lumen which give rise to the spermatozoa or male germ cell Adjacent to the basement membrane are found several layers of primitive germ cells or spermatogonia These are cuboidal cells and show active proliferation, which produces from every spermatogonium two primary spermatocytes and each of these in turn divides to produce two secondary spermatocytes Each secondary spermatocyte divides giving rise to two spermatids which are seen near the lumen of the tubule attached to the Sertoli cells the latter cells do not form part of the germinal epithelium From here sper

matids are removed to the epididymis where they are stored and during this term of storage undergo morphological changes which produce the spermatozoa—the male sex cell in its final form This cell has a head of 3 x 9 μ in diameter and a tail of some 45 μ in length

(ii) *Sertoli Cells* Between the germinal cells are seen a number of elongated columnar cells which are radially arranged from the basement membrane towards the lumen These are the Sertoli cells and they are believed to exert a supporting or architectural function as well as one of providing nourishment to the germinal epithelium especially the spermatids which remain attached to the luminal end of the Sertoli cells before they are removed to the epididymis Sertoli cells do not appear to multiply and are relatively few in number compared with the germinal cell population

Fig 18 The histology of the normal testis (*Maximow and Bloom*)

INTERSTITIAL TISSUE (Fig 18) The great importance of the interstitial tissue of the testis lies in the cells of Leydig which become conspicuous at the time of puberty They are large oval cells (15 20 μ) with a vesicular nucleus Leydig cells appear in clusters or nests which include small blood vessels situated in the angles between the folds of the tubules The most important inclusions within the cytoplasm of these cells are the crystalloids of Reinke These structures exhibit certain staining properties which are thought to be specific for Leydig cells being otherwise seen only within certain cells in the hilum of the ovary which are derived from the medulla of the indifferent gonad and are believed to be the female counterpart of Leydig cells Two generations of Leydig cells appear one just before birth and one at puberty These cells do not multiply and are thought to be formed by the transformation of mesenchymal cells[1]

SPERMATOGENESIS Spermatogenesis may be defined as the proliferation of spermatogonia together with the morphological and genetic changes which ultimately produce spermatozoa Like oogenesis, the female counterpart spermatogenesis involves three phases—proliferation growth and maturation (Fig 15)

(i) *Proliferation* This phase consists of multiplication of primitive spermatogonia by ordinary mitotic division, producing several generations of spermatogonia which are pushed nearer to the tubular lumen

(ii) *Growth* There follows a period of growth in which the young spermatogonia enlarge to form primary spermatocytes These cells still contain the 48 chromosomes which characterise all human somatic cells

(iii) *Maturation* Maturation involves two meiotic or reduction divisions The first gives rise to two secondary spermatocytes and involves the simple separation of each pair of chromosomes This division is called a reduction division and is qualitative since the two cells formed are genetically different The second division converts each secondary spermatocyte into two spermatids During this process each chromosome splits into two halves which are genetically identical In this case, the division is equational and quantitative since the two cells produced are genetically identical Spermatids now undergo a final stage of morphological development for which there is no equivalent in the process of oogenesis This process produces the spermatozoa but involves neither division nor genetic alternation it is called spermiogenesis

VARIATIONS IN TESTICULAR HISTOLOGY It is not proposed to discuss those disease processes which affect the histological appearance of the testis However it may be pointed out that the microscopic picture of one testis is not necessarily represented by a small histological section since spermatogenesis may be active in some parts and quiescent in others The great variations seen in testicular histology involve the germinal epithelium rather than the interstitial tissue Among the factors which affect the appearance of the germinal epithelium are age environmental temperature fever disturbances of general health exposure to radioactive agents and certain chemical poisons

DESCENT OF THE TESTIS The rapid growth of the foetus causes the gonads to adopt a more caudal position than that in which they originally develop During the 5th month of embryonic life a pouch of peritoneum extends along the inguinal canal into the developing scrotum which it comes to line Between the 7th and 9th months the testis descends behind this pouch and enters the scrotum in an extraperitoneal position The gland takes with it a blood nerve and lymph supply together with the ductus deferens—these structures bound together with connective tissue constitute the spermatic cord The testis becomes enfolded by peritoneum and the connection with the coelom closes The mechanism of this descent is not certain A fibrous band the gubernaculum testis joins the lower pole of the testis to the inner aspect of the scrotum It has been suggested that the gubernaculum pulls the testis into the scrotum by contracting It seems more probable however that the gubernaculum is merely a guide which enables the testis to find its way during descent [2]

Since the testis descends at puberty in some animals or descends only during the mating season in other species it has been suggested that descent is initiated by means of gonadotrophic hormones In the human it may be that maternal chorionic gonadotrophin crosses the placenta and causes descent of the testis Failure of the testis to descend could therefore result from some hormonal defect as yet not understood or in other cases it may be due to the fact that the testis follows the wrong path of descent owing to some anatomical peculiarity of the gubernaculum or of the inguinal canal

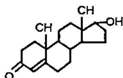
The Internal Secretions of the Testis

The testis is known to produce androgenic hormones and oestrogens have been found in the gland In addition a good deal has been written about the so called second testicular hormone

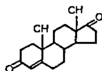
ANDROGENS An androgen may be defined as a substance which can stimulate the development and activity of the male accessory genital organs and secondary sexual characteristics

Source and Number It is probable that the testis produces more than one androgen but the most potent is testosterone which is generally regarded as the important testicular androgen in man Testosterone has been found in the testis of a number of animals including man and has been isolated from spermatic vein blood[14] In spite of certain evidence to the contrary most workers accept the Leydig cells as the source of testicular androgens In addition to testosterone it is thought that the human testis secretes Δ^4-androstene 3 17 dione

Chemistry Testicular androgens are steroids and therefore possess the basic structure common to such compounds



Testosterone



Δ^4 Androstene 3 17 dione

It will be seen that these two steroids possess a methyl group at C_{10} and that testosterone is not a 17 ketosteroid (since it lacks a C = O group at C_{17}) while Δ^4-androstene 3 17 dione is a 17 ketosteroid

Metabolism (a) *Anabolism* It has been suggested that chol esterol may be the source of androgens in the testis It may be that cholesterol is itself formed from acetate and then converted to testosterone[17]

(b) *Fate* Androgens are not stored in the body but are used or excreted A large proportion of injected androgen cannot be recovered from the urine because the catabolism of androgen pro duces a number of inactive metabolites

(c) *Catabolism* Testosterone is converted to androsterone and etiocholan 3α ol 17-one which are excreted in the urine Other urinary metabolites of testosterone include epiandrosterone and dehydroepiandrosterone Of these compounds androsterone dehydroepiandrosterone and epiandrosterone are physiologically active The site of these conversions is extratesticular since they can be demonstrated in eunuchs[15,16] It is probable that they take place in the liver where androgens are also rendered inactive by means of the process called conjugation[18] (page xiii)

(d) *Excretion*[14 15 16] Testosterone is not excreted as such unless administered in large doses It is converted to the four steroids mentioned above and these are conjugated in the liver in this conjugated or inactive form they appear in the urine The four known end products of testosterone metabolism are 17-ketosteroids so that estimations of urinary 17 ketosteroids give some indirect information about the rate of secretion of testos terone Some intestinal excretion of the products of androgen metabolism occurs probably by way of the bile

ACTION The action of testicular androgens will be regarded as the action of testosterone since nothing is known of the import ance and function of other testicular androgens under normal conditions In general this hormone causes an increase in blood flow and an acceleration of growth in the tissues upon which it acts Acceleration of growth involves an increase in the rate of synthesis of protein

(1) *Reproduction Accessory Genital Organs* Testosterone promotes the development and maintains the size of the male accessory genital organs both internal and external In the absence of testicular androgens these structures do not reach adult size or in the case of mature organs they slowly regress if the secretion of testicular androgens fails In short testosterone is responsible for that quantitative and qualitative development of the secondary sexual characteristics which distinguish man from boy In addition normal sexual behaviour and the regular occurrence of erections require among other things adequate concentrations of testos terone in the blood

The organs stimulated by testosterone include the scrotum which increases in size and develops rugae while the penis and prostate reach adult proportions

Body Hair (1) *Head* Testosterone initiates the recession of the male hair line which generally begins in the third decade (Fig 19) This is one of the features which distinguish the general appearance of a man from that of a boy Not all normal men show



Fig 19 The hair lines of a boy a woman and a man The hair line of the boy and that of the woman are essentially the same while that of the man shows temporal recession or calvity (*Fulton Textbook of Physiology*)

temporal recession but when present this sign is a good indication of adequate secretion of testicular androgens

(ii) *Face* The beard develops under the influence of testos terone which converts the soft down of the boy to the wiry bristles of the man Regression of the beard following castration in adult life is very slow

(iii) *Pubis and Axilla* Adrenocortical androgens stimulate the growth of pubic and axillary hair in women and castrates but the characteristic linear extension of the male pubic hair towards the umbilicus is due to the action of testosterone

(iv) *Body* Although testicular androgens may govern the dis tribution of hair on the trunk and limbs in a general sort of way the relationship is not close and genetic factors among others are important in determining the pattern of body hair

Spermatogenesis The presence of a certain concentration of testosterone in the blood is necessary for normal spermatogenesis (page 92)

Miscellaneous Among other secondary sexual characteristics testosterone is responsible for the deepening of the male voice at puberty the greater muscular development in men than in women and it plays some part in determining the distribution of body fat and the skeletal configuration of adult men Testosterone also causes the appearance of acne at puberty and is partly responsible for the awakening of the sexual instinct at that time The action of testicular androgens upon the breast varies in different species and is discussed elsewhere (page 132)

(2) *Metabolism*

(i) *Retention of Nitrogen* Testosterone causes the body to retain nitrogen this is associated with an increase in the synthesis of protein The accessory genital organs have first claim on the anabolic processes stimulated in this way The anabolic effect of injected testosterone is most marked in the absence of testicular tissue It appears to be possible to alter the chemical structure of certain androgens in such a way as to retain the protein anabolic effect at the expense of the androgenic activity although so far all the substances produced with this aim in view have either shown some androgenic properties or exhibit only weak effects upon protein anabolism In its anabolic actions testosterone is synergistic with growth hormone methyl testos terone brings about increased excretion of creatine but testos terone propionate does not

(ii) *Electrolyte and Water Balance* Testosterone causes retention of sodium chloride potassium inorganic phosphorus and water The effect of testosterone upon the retention of sodium chloride and water is weaker than that of adrenocortical mineralocorticoids

(iii) *Bone* Testosterone exerts the following important actions upon bone metabolism and linear growth —

(a) It promotes the deposition of protein matrix

(b) It causes retention of calcium and phosphorus

(c) It promotes the closure of the epiphyses

In this way testosterone stimulates the growth of bone and yet limits the final size attained by long bones because it promotes epiphysial closure

(iv) *Skin* Testosterone exerts three important actions upon skin

(a) It favours the deposition of melanin in the skin

(b) It increases the blood flow through the skin

(c) It stimulates sweat glands and sebaceous glands leading to the acne of youth

(v) *Blood* Testosterone stimulates the production of haemoglobin and red blood cells The importance of this action under normal conditions is unknown

(vi) *Larynx* Testicular androgens cause thickening of the laryngeal mucosa and an increase in the length of the vocal cords which gives rise to the deep voice of the adult male

(vii) *Kidney* Testosterone appears to stimulate growth in the kidney but the significance of this action is not understood[13]

(viii) *Behaviour* Testosterone causes aggressive behaviour in man and animals This is especially seen in animals at the time of mating and occurs in castrated male animals treated with testosterone

EXTRATESTICULAR SOURCES OF ANDROGENS The adrenal cortex is the only other organ which is known to produce androgens The idea that the ovary may produce androgens while possible remains unproven

ASSAY The most important method by which testicular androgens are estimated for clinical purposes is the examination of the 17 ketosteroid content of the urine This provides only an indirect measure of testicular hormone secretion in men since two thirds of the urinary 17 ketosteroids are adrenocortical in origin and

moreover not all androgens are 17 ketosteroids however gross abnormalities in 17 ketosteroid excretion indicate some disturbance of androgen secretion Bioassay of androgens may be performed using the change in the comb of the castrated cock (Fig 20) or increase in the weight of the seminal vesicles of the castrated cat or rabbit as the end point

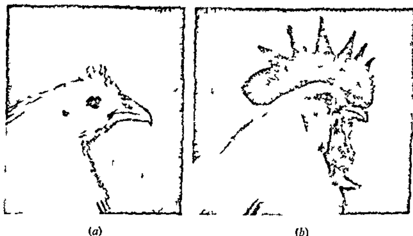


Fig 20 The effect of testosterone upon the comb of a cock (*a*) before treatment (*b*) after treatment (*Fulton* *Textbook of Physiology*)

METHYL TESTOSTERONE When the C_{17} of testosterone is methylated certain of the properties of the original compound are changed Most important of these changes is the absorption and activity of methyl testosterone taken by mouth This is thought to result from absorption from the alimentary tract through lymph channels thereby avoiding the liver When methyl testosterone is administered by mouth it does not increase the urinary excretion of 17 ketosteroids

Control of Testicular Function

Although there is reason to believe that testicular function may be influenced by and in turn may influence the behaviour of other endocrine glands (especially the thyroid and the adrenal cortex), it is directly controlled by the adenohypophysis In the absence of pituitary stimulation testicular function (germinal and endocrine) come to a halt The adenohypophysis owes its capacity to stimulate testicular function to the secretion of two hormones called follicle stimulating hormone (FSH) and interstitial cell-

stimulating hormone (ICSH) FSH stimulates the development of the germinal epithelium at puberty and enables normal spermatogenesis to continue during adult life in the absence of this trophic effect spermatogenesis is arrested at the stage of primary spermatocytes and can proceed no further ICSH on the other hand stimulates the cells of Leydig to produce testosterone in the absence of this trophic effect secondary sexual characteristics do not develop and the accessory genitalia remain infantile ICSH appears to be identical with the luteinizing hormone of the female

This relationship between the adenohypophysis and the testis is not one sided If both testes be removed the adenohypophysis increases in size and its beta cells become larger and more numerous These enlarged beta cells contain colloid and are called castration cells Again destruction of the germinal epithelium causes an increase in the production of FSH orchidectomy also promotes an increase in FSH production but it remains uncertain whether this procedure also causes an increase in ICSH secretion

These facts have suggested that the reciprocity which exists between the thyroid gland and the adenohypophysis for example may also exist between the testis and the pituitary gland However the observations quoted above are not in harmony with a simple relationship of that order If FSH stimulates spermatogenesis it would be expected that some hormone produced during this process would act upon the adenohypophysis to prevent or check the secretion of further FSH In this way FSH would stimulate the production of the very hormone which is to act as its own inhibitor such an arrangement would seem to make for a smooth and controlled relationship Again if ICSH promotes the production of testosterone the latter may in turn check the secretion of further ICSH Now it can be shown that both oestrogens and testosterone will depress the excessive production of FSH following orchidectomy (oestrogens more readily than testosterone) Unfortunately estimation of ICSH is very difficult and it is impossible to say what effect orchidectomy and testosterone have upon its secretion Such confusing observations have elicited three major theories which attempt to reconcile these inconsistencies and so bring the relationship between the testis and the pituitary into line with similar relationships between other target glands and the adenohypophysis

1) *Utilization Hypothesis* [9] One school believes that the secretion of FSH is controlled by the rate at which this hormone

is used by the germinal epithelium In other words spermato genesis in some way removes FSH from the blood and this fall in blood level is made good by the adenohypophysis which secretes more FSH to replace that which has been used

2) *A Second Testicular Hormone*[10] If the testis secretes a second hormone this may be produced by the germinal epithelium and could be responsible for controlling the secretion of FSH Supporters of this hypothesis call their hormone inhibin or X hormone [0] It must be remembered however that this hormone has never been isolated and is the result of an ingenious theory which would explain the relationship between the pituitary and the germinal epithelium

3) *Oestrogens* A third group of workers regard oestrogen as the second testicular hormone It is generally agreed that the testis does secrete oestrogens and there may be no need to postulate the existence of an X hormone This conception accords well with the state of affairs in the female where the secretion of FSH is controlled by the ovarian production of oestrogens

Some of these difficulties could be resolved if the source of testi cular oestrogens were known Here again we meet three different schools of thought One group [1,2] of workers believes that the Ser toli cells produce oestrogen Sertoli cell tumours occur in dogs and the affected animals show signs of excessive oestrogen secretion [1,2] A second group of workers can show that semen contains oestrogens and they suggest that spermatogenesis involves the production of oestrogens[23] A third group has produced con vincing data to support their contention that the Leydig cells secrete the testicular oestrogens [4]

At the present time these conflicting views cannot be resolved It may be suggested as a working hypothesis which is more in harmony with these observations than other ideas which have been put forward that ICSH stimulates the production of testos terone which in turn checks the secretion of ICSH a similar reciprocity exists between FSH and some product of the con tents of the seminiferous tubules (perhaps oestrogen)

INTERRELATIONSHIP BETWEEN THE TWO TESTICU LAR FUNCTIONS Although spermatogenesis and the secretion of androgens occur in some measure independently it has been shown that androgen secretion is necessary for normal spermato genesis [5,6] The germinal epithelium is such a vulnerable struc- ture that most noxious influences destroy its component cells before they affect the Leydig cells with the result that it is difficult

to study spermatogenesis in the absence of normal Leydig cell function However experimental work has shown that some androgen is necessary for normal germinal function and that very small quantities of androgen in excess of that required for this function may depress spermatogenesis by depressing the secretion of FSH by the adenohypophysis There is some evidence to show that gross excess of testosterone may have a deleterious effect upon the germinal epithelium by direct action [7,34]

The germinal and endocrine functions of the testis are together involved in the production and discharge of semen Erection and ejaculation depend upon nervous reflexes mediated through centres in the lumbosacral region of the spinal cord and also upon the secretion of androgens by the cells of Leydig The mechanism by which testicular androgens assist in these functions is not certain but in the absence of adequate blood levels of testosterone erection and ejaculation are usually quantitatively and qualitatively defective

Semen

Semen is the fluid secreted by the accessory genital glands of the male in which spermatozoa are suspended Normal fertile men produce semen containing between 20 and 200 million spermatozoa per ml of semen Spermatozoa leave the testis to be stored in the epididymis where they remain inert bathed in a little acid fluid the male sex cells are not stored in the seminal vesicles Seminal fluid is secreted by the seminal vesicles the prostate and the bulbo urethral glands Among the components of human seminal fluid fructose is characteristic and is added by the prostate gland where it is formed from blood glucose The prostate also adds acid phosphatase and citric acid whose functions are unknown together with calcium and fibrinolysin [9] Calcium is important in the coagulation of semen a change which is designed to prevent its escape from the female genital tract while fibrinolysin causes the subsequent liquefaction which allows the spermatozoa to penetrate further into the uterus The seminal vesicles add phosphorylcholine to the semen which is important as the substance detected by a well known forensic test for semen The spermatozoa themselves contain the enzyme hyaluronidase[30] which is thought to assist in penetrating the gel which surrounds the ovum Spermatozoa remain inert until they are suspended in the seminal fluid when they exhibit the characteristic motility which enables them to reach the ovum It should

is used by the germinal epithelium In other words spermato genesis in some way removes FSH from the blood and this fall in blood level is made good by the adenohypophysis which secretes more FSH to replace that which has been used

2) *A Second Testicular Hormone*[20] If the testis secretes a second hormone this may be produced by the germinal epithelium and could be responsible for controlling the secretion of FSH Supporters of this hypothesis call their hormone inhibin or X hormone[20] It must be remembered, however that this hormone has never been isolated and is the result of an ingenious theory which would explain the relationship between the pituitary and the germinal epithelium

3) *Oestrogens* A third group of workers regard oestrogen as the second testicular hormone It is generally agreed that the testis does secrete oestrogens and there may be no need to postulate the existence of an X hormone This conception accords well with the state of affairs in the female, where the secretion of FSH is controlled by the ovarian production of oestrogens

Some of these difficulties could be resolved if the source of testi cular oestrogens were known Here again we meet three different schools of thought One group [1,2] of workers believes that the Ser toli cells produce oestrogen Sertoli cell tumours occur in dogs and the affected animals show signs of excessive oestrogen secretion [1,2] A second group of workers can show that semen contains oestrogens and they suggest that spermatogenesis involves the production of oestrogens[23] A third group has produced con vincing data to support their contention that the Leydig cells secrete the testicular oestrogens [4]

At the present time these conflicting views cannot be resolved It may be suggested as a working hypothesis which is more in harmony with these observations than other ideas which have been put forward that ICSH stimulates the production of testos terone which in turn checks the secretion of ICSH a similar reciprocity exists between FSH and some product of the con tents of the seminiferous tubules (perhaps oestrogen)

INTERRELATIONSHIP BETWEEN THE TWO TESTICU LAR FUNCTIONS Although spermatogenesis and the secretion of androgens occur in some measure independently it has been shown that androgen secretion is necessary for normal spermato genesis [5,6] The germinal epithelium is such a vulnerable struc- ture that most noxious influences destroy its component cells before they affect the Leydig cells with the result that it is difficult

other parts of the autonomic nervous system and produce reactions in other viscera, e g tachycardia and increased vasomotor activity Some afferent impulses from the lumbosacral centre presumably reach the cerebral cortex, but much of the sensation associated with orgasm is thalamic During ejaculation the accessory glands discharge their secretions in the following order —prostate seminal vesicles and bulbo-urethral glands[33]

REFERENCES

1 Everett N B Biol Rev 20 45 1945
2 Politzer G Leitscher f d ges Anat 87 766 1928
3 Heys F Quart Rev Biol 6 1 1931
4 Arey L B Developmental Anatomy Saunders Philadelphia 1948
5 Witschi E J Esper Lool 52 235 1929 58 113 1931 9 460 1934
6 Moore C R J Clin Endocrinol 10 942 1950
7 Greene R R J Clin Endocrinol 4 335 1944
8 Jost, A Recent Progress in Hormone Research 8 379 1953
9 Moore C R et al Surg Gyn & Obst 96 641 1953
10 Marberger E & Nelson W O J Clin Endocrinol 14 768 1954
11 Hooker C W Recent Progress in Hormone Research Academic Press N Y 3 173 1948
12 Burrows H Brit J Surg 23 658 1936
13 Engle E T Endocrinology 16 513 1932
14 West C O et al J Clin Endocrinol 12 915 1952
15 Mason H L J Biol Chem 162 745 1946
16 Marrian G F Nature 154 19 1944
17 Perlman M L Endocrinology 46 341 1950 46 347 1950
18 West C D et al J Clin Endocrinol 11 897 1952
19 Heller C G et al Ann N Y Acad Sci 55 685 1952
20 Howard R P et al J Clin Endocrinol 10 121 1950
21 Teilum G J Clin Endocrinol 9 301 1949
22 Huggins C & Moulder P V Cancer Research 5 510 1945
23 McCullagh E P & Schaffenberg C A Ann N Y Acad Sci 55 674 1952
24 Maddock, W O & Nelson W O J Clin Endocrinol 12 985 1952
25 Walsh L et al Am J Physiol 107 508 1935
26 Smith P E Yale J Biol & Med 17 281 1944
27 Ludwig D J Endocrinology 46 453 1950
28 Blundau R J Amer J Anat 77 253 1945
29 Higgins C Physiol Rev 25 281 1945
30 Chang M C Annals N Y Acad Sci 52 1192 1950
31 Kriss F Z Anat Emo Gesch 61 455 1921
32 Semans J H & Longworth O R J Urol 40 836 1938

be pointed out however that spermatozoa removed by needle from the epididymis assume motility at once and that seminal fluid is not essential to fertility [8] The final phases of reproductive physiology in the male consist of erection and ejaculation

Erection

The phenomenon of erection is vascular and consists of processes which increase the volume of blood entering the penis and processes which diminish the volume of blood leaving the organ The former consist of arterial dilatation The intima of the arteries of the penis show longitudinal ridges which partly occlude their lumina Erection begins with arterial dilatation[31] Escape of blood is impeded by funnel like valves in the larger veins and is for the most part a passive process resulting from the effect of increased pressure on the penile veins Return to the resting state is initiated by arterial constriction and gradual escape of blood, which lowers the pressure in the vascular spaces and so aids the venous drainage which proceeds at an ever increasing rate

In the corpora cavernosa the large venous spaces are seen towards the centre while the reverse is true of the corpus spongiosum which surrounds the urethra In this way the urethra remains unoccluded during erection Arterial dilatation and erection follow stimulation of the pelvic splanchnic nerves (nervi erigentes) while sympathetic nerve stimulation produces the opposite effect

Ejaculation

The act of ejaculation is a double one and consists firstly, of the contraction of the smooth muscle of the internal genital organs which delivers semen into the urethra (emission) and secondly, the expulsion of semen from the urethra as a result of contraction of the bulbocavernosus muscle (ejaculation proper) The whole process is essentially a reflex Afferent impulses arise in the glans penis and are transmitted to the spinal cord by the internal pudendal nerves these nerves have their origin in segments S1 and S2 Efferent impulses arise from the upper lumbar segments and are distributed by way of the hypogastric nerves and the hypogastric plexus to the accessory genitalia these nerves are responsible for emission Ejaculation is controlled by the internal pudendal nerves which convey parasympathetic impulses from S1 and S2 These mechanisms are integrated in an ejaculatory centre in the lumbosacral cord[3] The impulses associated with ejaculation spread to

CHAPTER V

THE OVARY

Introduction

The female gonad directs the reproductive life of woman It does this by means of three interrelated functions In the first place the gland produces the female sex cell or ovum Secondly the ovary secretes a group of hormones called oestrogens which so condition the accessory genital organs as to favour the fertilization of the ovum and which produce the secondary sexual characteristics of woman Thirdly the ovary secretes a hormone called progesterone which prepares the mucous membrane of the uterus for the reception and nutrition of the fertilized ovum

The basis of female reproductive life consists of a series of cycles during which one ovum is produced and preparations are made within the uterus to receive the expected zygote When the ovum is not fertilized the elaborate mucosa of the uterus is shed and the whole cycle begins again

Embryology

The early development of the urogenital system has already been discussed (page 75) In the female the indifferent gonad becomes an ovary and the Wolffian duct atrophies in favour of the Mullerian duct which produces a large part of the female genital tract

DIFFERENTIATION OF THE OVARY During the seventh week changes occur in the indifferent gonad which indicate that it is destined to become an ovary The primary sex cords (page 79) disappear leaving only a few remnants of the hilum They are replaced by the secondary sex cords which grow in from the germinal epithelium The cellular proliferation associated with these changes produces a secondary cortex while any sex cells in the primary cortex or in the medulla disappear The secondary sex cords give rise to the oogonia or primitive sex cells of the secondary cortex The oogonia become surrounded by epithelial cells thereby forming primary follicles (Fig 21) These epithelial cells are thought to arise from ovarian mesenchyme and will later

33 Higgins C & McDonald D F J Clin Endocrinol 5 226 1945
34 Henderson E et al J Clin Endocrinol 8 851 1948
35 Heller C G et al Fertility and Sterility 1 415 1950
36 Goldschmidt R B Theoretical Genetics p 424-475 Berkeley University of California Press 1955
37 Witschi E Recent Prog Hormone Res 6 1 1951

become the granulosa cells The ovary like the testis descends into the pelvis being checked in this descent by the attachment of its gubernaculum to the uterus

GENITAL DUCTS The Mul erian duct develops as a groove on the ventrolateral aspect of the urogenital fold during the 6th week of development (Fig 22) The groove is converted into a tube and the two Mullerian ducts fuse in the midline (Fig 23) The united lower portions of the Mullerian ducts form the uterus and the upper two-thirds of the vagina while the upper segments form the fallopian tubes Meanwhile the Wolffian duct degenerates leaving only vestigial remnants as evidence of its existence (Fig 24)

Histology

Between puberty and the menopause the histology of the ovary undergoes cyclical changes Every month one or other ovary (usually alternately) produces a female sex cell or ovum The gland has a cortex and medulla the whole being covered by a germinal epithelium (except at the hilum) which is continuous with the peritoneal mesothelium In the cortex which occupies between half and two thirds of the ovarian substance numerous ovarian follicles are seen at various stages of development (Fig 21)

OVARIAN FOLLICLES At birth the ovarian cortex contains between 40 000 and 400 000 primary follicles of which less than 400 will ever mature The majority of follicles degenerate so that within a few years of the menopause none remain Each follicle consists of a large ovum surrounded by several layers of flat epithelial cells called the membrana granulosa It is uncertain whether the germ cells present at birth constitute the sole source of ovarian follicles, or whether they may receive later additions from the germinal epithelium

From the time of puberty certain primary follicles develop and some of these reach maturity with the result that during the reproductive life of a woman the ovary contains follicles in all stages of growth and development Growth of a follicle is accompanied by the proliferation and stratification of the surrounding granulosa cells and an increase in the size of the ovum together with the formation of a connective tissue capsule (Fig 21) As the follicle grows it pushes deep into the substance of the ovary and soon a cavity or antrum appears within the follicle which is now called a vesicular follicle The antrum comes to contain a

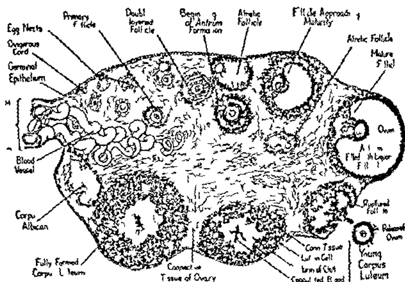


Fig 21 Schematic drawing of the ovary showing the various stages in the natural history of an ovum and its follicle The figure should be followed in a clockwise direction (*From Patten Human Embryology Courtesy of the Blakiston Company*)

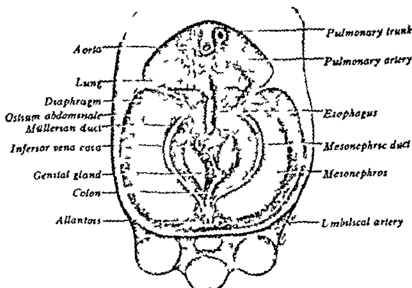


Fig 22 Ventral dissection of a pig embryo to show the developing Mullerian ducts (*Arey*)

around the follicle and taking on an epithelioid appearance these cells together constitute the theca interna (Fig 21) Outside the theca interna a second layer of ovarian stroma grows around the follicle to form the theca externa

The follicle now grows towards the surface of the ovary and in its mature form is called the Graafian follicle when it occupies the entire width of the cortex eventually producing a slight bulge on the surface of the ovary called the stigma It is through this thin avascular area that the follicle will eventually rupture and expel the contained ovum during the process of ovulation The ovum surrounded by the cells of the discus proligerus reaches a size of 150 μ which makes it just visible to the naked eye Fertilization generally takes place near the fimbriated end of the tube and it is believed that the ovum can survive for about 24 hours before it is either fertilized or dies

CORPUS LUTEUM After ovulation the remainder of the follicle collapses and becomes converted into the corpus luteum (Fig 21) The granulosa cells enlarge and come to contain a yellow pigment which gives the corpus luteum its name These cells are now called the granulosa lutein cells and in multiplying they produce folds of redundant tissue about the follicular cavity which has now been sealed off Meanwhile delicate vessels penetrate the corpus from the surrounding thecal layers and the cavity may become filled with blood[1] Cells from the theca interna migrate along these vessels and taking on an epithelioid appearance they come to contain fatty droplets they are called the theca-lutein cells The granulosa lutein cells and the theca lutein cells between them secrete progesterone the hormone of the corpus luteum The corpus reaches maturity about 7 days after ovulation and about 7 days before the following menstrual period (Fig 25) In the absence of fertilization the corpus begins to disintegrate shortly before the next menstrual period Should fertilization occur however the corpus develops instead of regressing and remains active until the second half of gestation

ATRESIA OF FOLLICLES During each cycle a number of follicles begin to mature but only one is destined to ovulate The remainder undergo a destructive process called atresia which in the case of primary follicles is quickly accomplished leaving no trace of their existence but is slower and less complete in the case of vesicular follicles Larger follicles failing to undergo complete atresia may give rise to small cysts

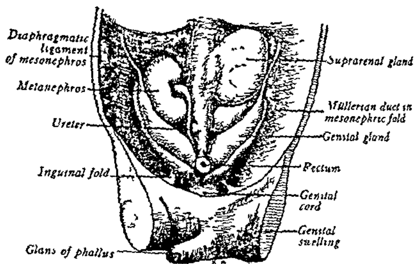


Fig 23 Ventral dissection of a human foetus of eight weeks to show the Mullerian ducts which have fused in the midline (*Arey*)

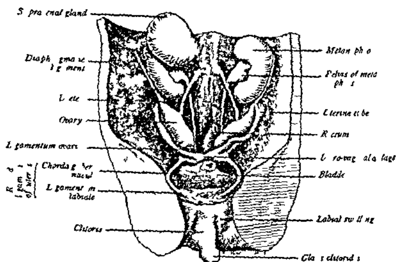


Fig 24 A female foetus of nine weeks showing the development of the genital ducts (*Arey*)

fluid (liquor folliculi) in which oestrogenic hormones are to be found The ovum gradually adopts an eccentric position within the follicle and is surrounded by layers of granulosa cells called the discus proligerus A layer of connective tissue cells condenses

Accessory Genital Organs

The secretions of the ovary influence the structure and function of the accessory genital organs The uterus is lined by a highly specialised mucous membrane called the endometrium This mucosa undergoes a cycle of structural modification which keeps step with the ovarian cycle The mucous membrane of the vagina also shows cyclic activity but this is far less striking than the changes which affect the endometrium

THE ENDOMETRIUM The endometrial cycle begins with the reconstitution of the uterine mucosa after menstruation this leads to the proliferative or follicular phase which reaches its peak at the time of ovulation Following the proliferative phase further elaboration of the mucosa occurs in time with the activity of the corpus luteum this is called the secretory or luteal phase In the absence of fertilization menstruation occurs and the elaborate mucosa goes to waste (Fig 25) The endometrium consists of a specialised type of connective tissue the stroma covered by a layer of columnar epithelium which is continuous with the many tubular glands which penetrate the mucous membrane The stroma is composed of connective tissue cells embedded in a delicate supporting tissue which contains capillaries arterioles venules and arterio venous anastomoses these structures vary in appearance and activity during the different phases of the cycle The mucosa rests directly upon the myometrium and consists of three layers—a narrow basalis which does not participate in the cyclical changes to be described the thick spongiosa which constituting the bulk of the endometrium lies directly upon the basalis and a superficial narrow, dense zone called the compacta The spongiosa and the compacta together constitute the functionalis so called because together they take part in the cyclical changes to which the endometrium is subjected

The blood supply of the endometrium is specialised and adapted to the periodicity of the mucosa[3,4] It consists of two systems of arteries derived from the arcuate branches of the uterine arteries As the nutrient arteries approach the endometrium each gives rise to a short straight basal artery which supplies the basalis and a coiled spiral arteriole which enters the functionalis In this way the fixed basalis and the constantly changing functionalis each possess an independent blood supply

Proliferative Phase Epithelial regeneration begins during the terminal stages of menstrual shedding and this phase of endometrial reconstruction continues until ovulation that is to say for

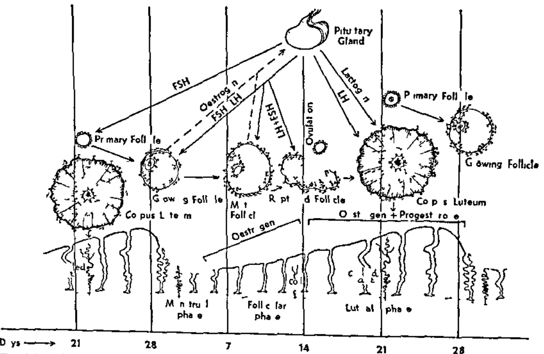


Fig 25 Schematic representation of the pituitary ovarian and endometrial cycles In the case of the ovarian cycle three generations of follicles are shown On the left is seen the corpus luteum associated with the first menstrual phase above this are represented the various stages in the development of the follicle associated with the second menstrual phase On the right the early stages of development of a follicle of the third generation are seen these will be associated with the next menstrual cycle Lactogen is an alternative name for prolactin

ning of secretory activity The glands now become tortuous while the mucous membrane reaches a width of about 3 mm

Secretory Phase (Fig 27) Merging imperceptibly with the last stages of proliferative activity the secretory phase ends a few days before the next menstrual period (Fig 25) This phase is marked by increased vascularity oedema and secretory activity of the glands The latter become wider and more tortuous with sacculation in their deeper reaches The nuclei of the endometrial cells return to the base of the cell and displace the secretory vacuole towards the lumen These vacuoles contain glycogen and mucin Meanwhile the spiral arterioles grow nearer to the surface of the endometrium and the thickness of the stroma is increased as the result of oedema The stromal cells now contain much more cytoplasm than before[1] The three layers of the endometrium are now clearly defined and the whole structure attains a width of 6 mm



Fig 27 The endometrium in the secretory phase (*Novak*

a period of approximately 14 days (Fig 25) The basalis is quickly covered with low cuboidal epithelium and short straight glands descend into the deepest reaches of the mucosa The stroma at this stage is dense and avascular reaching a thickness of about 1 mm (Fig 26)



Fig 26 The endometrium in the proliferative phase (*Novak Menstruation and Its Disorders D Appleton Century Co*)

Proliferation of stroma and mucosa produces longer glands lined by taller cells and the spiral arterioles grow into this newly developed mucosa becoming more tightly coiled perhaps because they grow more rapidly than the mucous membrane In the epithelial cells subnuclear vacuoles appear and mark the begin

bodies (Fig 15) Secondly there occurs during oögenesis nothing which corresponds to the elaborate morphological changes which convert a spermatid into a spermatozoon (page 84) During oogenesis the same chromosomal reduction occurs by meiosis except that the daughter cells always contain an X chromosome (Fig 15) As in the case of spermatogenesis oögenesis consists of three phases proliferation growth and maturation

Proliferation The period of proliferation occurs before birth and gives rise to primitive oogonia

Growth Growth of oogonia begins at puberty under the influence of pituitary gonadotrophins and produces primary oocytes

Maturation The first maturation division produces a secondary oocyte and a polar body The second division produces a mature ovum and the second polar body (page 84) There is no general agreement as to whether this last division occurs before or after ovulation[2] The first of these changes involves the simple division of each pair of chromosomes and is therefore reductional and qualitative—reductional in that the amount of chromosomal substance is reduced by half and qualitative in that each of the two nuclei produced contains a different genetic pattern The division of the nuclear chromatin is equal but the cytoplasm is so divided that the polar body receives only a small fraction the rest going to the secondary oocyte Thus the secondary oocyte comes to contain 24 chromosomes

In the second division each single chromosome splits and one half passes to each of the two daughter cells This division is described as being equational and quantitative—equational because of the manner of chromosomal division and quantitative in that the two halves produced are genetically identical This second division resembles a mitotic division in that each chromosome is split into two equal halves Again the division of the cytoplasm is unequal and the mature ovum contains 24 chromosomes of which one is an X chromosome

OVULATION The immediate factor which precipitates ovulation is still uncertain It is generally held that the rapid outpouring of LH from the adenohypophysis is an important factor in initiating ovulation Some workers have suggested that a critical level of $\frac{FHS}{LH}$ concentration in the blood is the determining factor How important increasing tension of the fluid within the follicle may be is uncertain The rupture of the follicle usually occurs about the fourteenth day after the onset of menstruation

In time with the regression of the corpus luteum there occur regressive changes in the endometrium The stroma shrinks as the oedema subsides and there occurs a dense infiltration by poly morphonuclear cells The spiral arterioles become kinked thereby producing obstruction to the flow of blood This stasis leads to degenerative changes in the endometrium these are accentuated by the opening of arterio venous anastomoses, which cause the blood to by pass the mucosa These changes result in varying degrees of pallor and cyanosis, due to ischaemia and to stasis respectively

Menstrual Phase Menstrual bleeding lasts about four days and involves shedding of the spongiosa and compacta[6] The basalis remains intact because it possesses an independent blood supply Anoxia of the mucosa produces degeneration and rupture of the vessel walls while bleeding is checked by constriction of the spiral arterioles near the myometrium Periodic relaxation of arteries allows further bleeding and alternate dilation and con striction occur in different vessels at different times[5 7] In this way, each arteriole bleeds for about 30 seconds and does so only once during a given cycle The menstrual discharge attains a vol ume of between 50 and 150 ml it contains cervical mucus cell debris, endometrial secretion blood plasma and a fibrinolytic agent which prevents clotting

THE VAGINA The vagina undergoes cyclic changes in woman although they are less clearly defined and their functional significance less certain than those which occur in the endo metrium[8 9] The most striking change in vaginal histology is the increase in the number of cornified cells due to the action of oestrogens (Fig 29)

Production and Release of the Mature Ovum

Although the ovary contains germinal epithelium which is cap able of producing the mature female sex cell this is only accom- plished after a series of important genetic changes which together constitute the process of oogenesis The mature ovum is finally released from the ovary into the fallopian tube as a result of the process called ovulation

OÖGENESIS The changes which convert a primitive oogonium into a mature ovum constitute oogenesis Apart from two features oogenesis is strictly comparable with spermatogenesis (page 84) Firstly, each primitive oogonium produces only one mature ovum the three remaining products of oogenesis being functionless polar

Fig 29 A vaginal smear at the time of ovulation showing the characteristic clean appearance (*Novak*)

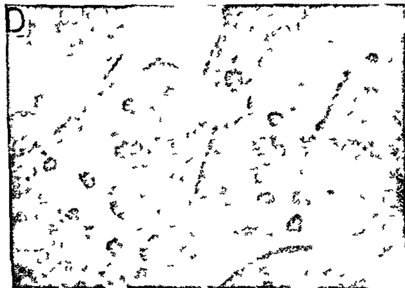


Fig 30 A vaginal smear during the luteal phase of the cycle showing invasion by numerous leucocytes The epithelial cells are clumped and their edges are folded (*Novak*)

CLINICAL EVIDENCE OF OVULATION

1) *Basal Temperature* The body temperature shows variations which coincide with events in the menstrual cycle Just before ovulation the temperature graph shows a sharp drop followed by a rise The subsequent plateau continues until just before the onset of menstrual bleeding when the temperature again falls, these changes provide indirect evidence of ovulation (Fig 28)

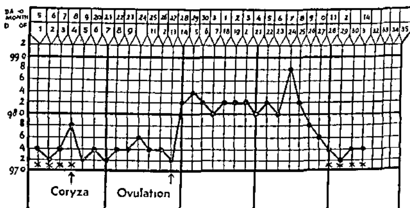


Fig 28 Temperature chart showing a fall and rise at the time of ovulation (*L J Soffer*)

2) *Endometrial Biopsy and Vaginal Smears* These investigations provide evidence of the action of oestrogens and later in the cycle they indicate the presence of progesterone which is indirect evidence that ovulation has taken place (Figs 26 30)

3) *Urinary Pregnanediol* Pregnanediol a product of the metabolism of progesterone appears in the urine at the time of ovulation and its concentration continues to rise thereafter The presence of increasing concentrations of pregnanediol in the urine indicates that ovulation has occurred and that a secreting corpus luteum has developed

4) *Cervical Mucus* Cervical mucus will leave a crystalline pattern on a clean dry slide if adequate secretion of oestrogen has occurred This test does not indicate whether ovulation has occurred or not

EVIDENCE OF A FUNCTIONAL CORPUS LUTEUM

Evidence of a functional corpus luteum provides evidence of previous ovulation since the former cannot exist unless the ovary has discharged an ovum

C_{17} and oestriol has three hydroxyl groups (3 16 and 17) Recently a fourth oestrogen (16-hydroxyoestrone) has been found in human urine

METABOLISM (a) *Anabolism* Little is known of the synthesis of oestrogens but it is thought that cholesterol may represent the precursor of these hormones It has also been suggested that certain androgens may act as oestrogen precursors[11] The classical experiments of Emmens[12] may throw some light on the anabolism of oestrogens He divided the vagina of the castrated mouse into two separate pouches in such a way that the upper pouch could be approached suprapubically A very small dose of the known oestrogens applied locally caused an oestrus response in the upper pouch but if the dose were small enough the lower pouch was unaffected Certain other compounds (which he called proestrogens) caused a reaction in both pouches or in neither because they must first enter the general circulation that they may be activated This activation appears to take place in the liver[1] In this way it has been shown that a number of steroid substances possess proestrogenic properties but the normal pathway of oestrogen synthesis is still uncertain

(b) *Catabolism* Oestrogens are inactivated in the liver by conjugation and are excreted in the urine in this conjugated form (page xiii) As far as is known, there is no renal threshold for oestrogens In order that it may successfully inactivate oestrogens the liver requires an adequate supply of vitamin B complex (chiefly thiamine and riboflavine) These facts were clearly demonstrated by the experiments of Biskind and Biskind[3 14] These workers implanted pellets of oestrone in the spleen of the castrated mouse no oestrus effects were observed in the vagina However when a new blood supply to the spleen was established through the phrenic vessels and the splenic artery and vein ligated thereby diverting the venous drainage of the spleen away from the liver continuous oestrus effects resulted Liver poisons (e g carbon tetrachloride) or diets inadequate in vitamin B complex produced the same result even with a patent splenic vein These experiments are taken to indicate that oestrogens are inactivated by normal liver tissue but that when these hormones enter the general circulation directly without first passing through the liver or when they pass through a liver whose cells have been damaged by poisons they are free to act upon their target organs

In addition to this power of conjugation the liver excretes free oestrogens in the bile in which they are conveyed to the small

1) *Endometrial Biopsy* Endometrial biopsy taken within 12 to 18 hours of the onset of menstrual bleeding shows the secretory activity typical of the action of progesterone if a functional corpus luteum be present (Figs 26 and 27)

2) *Vaginal Smear* It is frequently possible to find evidence of progesterone activity by examining a vaginal smear (Figs 29 and 30) Such smears show leucocytic infiltration and the epithelial cells are clumped with folding of their edges

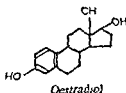
THE HORMONES OF THE OVARY

The ovary secretes two hormones, or rather two groups of hormones—oestrogens and progesterone

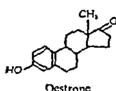
OESTROGENS Nature An oestrogen may be defined as a substance which is capable of inducing vaginal cornification in mice, identical with the changes of natural oestrus The word oestrogen does not indicate a specific chemical compound but is the generic name applied to a group of substances Urine (of both sexes) contains three oestrogens—oestradiol, oestrone and oestriol the last being peculiar to the human species Some workers believe that the hormone of the ovary is oestradiol and that the other two compounds represent less active metabolic products of oestradiol other authorities hold the view that the ovary secretes all three hormones

Source Oestrogens are secreted by the ovary and the placenta Small amounts are also produced by the adrenal cortex and the testis In the ovary the theca interna is probably the main source of oestrogens while the granulosa lutein and theca lutein cells *account for the secretion of oestrogens after ovulation It* appears that the granulosa and interstitial cells of the ovary are not important as sources of oestrogens[10]

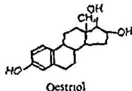
Chemistry The oestrogens secreted by the ovary are steroids compared with androgens they are less saturated and lack a methyl group at C_{10}



Oestradiol



Oestrone



Oestriol

It will be seen that oestradiol is so named because it has two hydroxyl (OH) groups oestrone has a ketone group (C = O) at

ACTION UPON REPRODUCTIVE PHYSIOLOGY

Uterus (a) *Endometrium* Oestrogens produce the histological picture described as the proliferative phase of endometrial activity (Fig 26) This involves thickening of the mucous membrane an increase in the size of the glands and changes in the arterioles (page 104) Prolonged secretion or administration of oestrogens brings about endometrial hyperplasia while the sudden withdrawal of oestrogens from the blood leads to bleeding The action of oestrogens upon the endometrium varies with the concentration of these hormones in the blood If the total concentration of oestrogens within the blood be below a certain critical level the endometrium does not develop the features characteristic of the proliferative phase and uterine bleeding cannot occur even when such oestrogens as are present be withdrawn Higher levels of circulating oestrogens permit endometrial proliferation and menstrual bleeding However above a second critical level oestrogens prevent bleeding this property forms the basis for the use of these hormones to stop excessive menstrual bleeding

(b) *Myometrium* Oestrogens stimulate uterine muscular activity and promote rhythmic contractions which may be important in the transport of sperms The same hormones also bring about enlargement of the uterus at puberty and during pregnancy

Vagina Oestrogens produce growth of vaginal epithelium with thickening and cornification (Fig 29) They also bring about the deposition of glycogen in the epithelial cells it is this glycogen which decomposes to lactic acid and this in turn is responsible for keeping the pH of the vagina at about 4 5 Oestrogens also bring about an increase in the volume of the vagina at puberty

Fallopian Tubes Oestrogens stimulate the development of the mucosa and the muscular activity of the fallopian tubes

Ovaries While small doses of oestrogens are essential for follicle maturation larger doses may inhibit ovarian function by depressing the secretion of trophic hormones by the adenohypophysis

Breast Oestrogens stimulate the development and pigmentation of the areolae together with the hypertrophy of the female breast which is characteristic of puberty During adult life these hormones stimulate the growth and development of ducts but in the human species appear to exert little action upon the alveolar pattern of the gland Oestrogen withdrawal at parturition may be a factor in initiating lactation

intestine and subsequently returned to the liver via the portal circulation This pathway of oestrogenic excretion and absorption is called the entero hepatic circulation[15]

It has been shown moreover that about two thirds of the oestrogens present in plasma are bound to protein and that protein bound oestrogen is in equilibrium with free oestrogen[16] this protein binding occurs in the liver Folic acid appears to be necessary for oestrogen activity in the tissues while recent studies suggest that the action of these hormones upon target organs may be related to the metabolism of β glucuronidase[17] Oestradiol is broken down to oestrone in the following way —[18]



OESTRADIOL $\rightleftharpoons$ OESTRONE $\rightarrow$ OESTRIOL

(c) *Storage and Excretion* Oestrogens are not stored in the body About 5 per cent of blood oestrogen is excreted in the urine and 5 per cent in the faeces (partly by biliary excretion and partly by direct intestinal excretion) The remainder is inactivated (chiefly in the liver) and excreted in conjugated form Three methods of inactivation of oestrogens within the body are known

(i) Transformation to inactive products of unknown composition
(ii) Conjugation with the formation of less active products
(iii) Transformation to inactive isomers

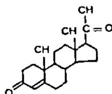
During the normal menstrual cycle two peaks of oestrogen *secretion appear One occurs at the time of ovulation and the* other during the luteal phase Low levels of oestrogen excretion involve the equivalent of 10 μg of oestrone per 24 hours, while high levels may reach 70 μg[21]

Assay Before it is possible to establish the concentration of oestrogens in urine this must first be subjected to a process of extraction which may involve vigorous chemical treatment this treatment destroys some of the oestrogenic activity of the urine Most of the chemical methods of assay are based upon the Kober reaction *which involves the formation of a pink colour when* phenolsulphonic acid acts upon oestrone[19] Bioassay methods are usually based upon the changes in the vaginal smear originally described in the Allen Doisy technique[20]

ACTION Oestradiol is the most potent of the three natural oestrogens and oestriol the weakest relative potencies vary according to the particular test used In general oestrogens bring about the development of the internal and external accessory genital organs and the appearance of the secondary sexual characteristics *by which woman is distinguished from girl*

PROGESTERONE Progesterone is secreted by the corpus luteum (probably from the theca lutein cells but perhaps also from the granulosa lutein cells) In addition progesterone is produced by the adrenal cortex and the placenta

Chemistry The hormone is a steroid



Progesterone

The structure of progesterone resembles that of androgenic hormones rather than oestrogens in that it shows one double bond in the A ring and two methyl side chains

Metabolism (a) *Anabolism* The fact that the adrenal cortex produces progesterone from cholesterol suggests that the latter may be the source of ovarian progesterone[25] Some workers believe that deoxycorticosterone may be a precursor of progesterone

(b) *Catabolism* Progesterone is not stored in the body it is either used or excreted and therefore the concentration in which it is found in blood is very low[28]

(c) *Excretion* Progesterone is not excreted as such One of its chief metabolic products, which is excreted in the urine is pregnanediol About 10 per cent of exogenous progesterone can be recovered from the urine in this form during the first trimester of pregnancy this percentage rises to about 30 Another product of progesterone metabolism found in the urine is pregnenolone these two compounds pregnanediol and pregnenolone are together called the pregnanediol complex Deoxycorticosterone is also a product of progesterone metabolism [6] The breakdown of progesterone to these metabolites probably occurs in the liver[18] During the follicular phase of the menstrual cycle pregnanediol is absent from the urine and first makes its appearance at about the time of ovulation During the luteal phase it reaches concentrations of between 2 and 8 mgm per 24 hours It appears in the urine conjugated at C_3 in the form of sodium pregnanediol glucuronide both free and conjugated pregnanediol are physiologically inactive

Action The essential function of progesterone as its name implies is to prepare the endometrium for the reception of the

METABOLISM

1) *Bone*[22] Oestrogens exert the following actions upon bone

(i) They bring about the development of the characteristic feminine skeletal contour—especially the appearance of the broad pelvis

(ii) They inhibit the laying down of matrix by osteoblasts and the growth of long bones

(iii) They cause maturation of centres of ossification and epiphysial closure

(iv) They cause calcium retention within the body

Oestrogens therefore inhibit bone growth and at the same time set a limit to such growth by promoting epiphysial closure

2) *Water and Mineral Metabolism*[23 24] Oestrogens cause water retention together with retention of nitrogen phosphorus calcium and sodium They are protein anabolic hormones

3) *Skin*[23 24] Oedema of the skin is a feature of oestrogenic activity and the extreme response is seen in the development of the sex skin of monkeys Diminution of sebaceous activity is also brought about by the action of oestrogens

4) *Bone Marrow* Large doses of oestrogens depress bone marrow activity [3 24] but this response is not seen with physiological doses

5) *Blood Vessels* Oestrogens cause vasodilatation apart from the turgescent effect which they exert upon the sexual organs [3 24]

Miscellaneous Actions Oestrogens are thought to encour ge the growth of certain malignant tumours and in some animals they bring about loosening of the pelvis at the pubic symphysis during pregnancy as a result of which the birth canal becomes wider

SYNTHETIC OESTROGENS A number of synthetic com pounds which are not steroids have been found to exhibit oestro genic effects in man and animals *The most important of these is* stilboestrol which is active when taken by mouth In addition to synthetic oestrogens organic chemists have made changes in the molecules of natural oestrogens which have produced useful phar macological properties One of the most important of these syn thetic oestrogens is ethinyl oestradiol which is oestradiol with an ethinyl group(*H—C≡C—*) at C_{17} *This compound is a powerful* oestrogen active by mouth and of low toxicity

ANDROGENS It is uncertain whether the ovary produces an androgenic hormone

Control of Ovarian Function

The chief controlling influence of the ovary resides in the secretion of gonadotrophic hormones by the adenohypophysis and the interaction between these hormones and those secreted by the ovary This is illustrated by the fact that ovarian and endometrial activity come to a standstill when the adenohypophysis is destroyed or removed It is now established that this function of the pituitary gland depends upon three hormones—follicle stimulating hormone (FSH) luteinizing hormone (LH) and prolactin FSH promotes the development of the Graafian follicle with its contained ovum LH acts synergistically with FSH to produce oestrogen secretion and ovulation while in addition it stimulates the luteinization of the theca and granulosa cells from which progesterone is secreted Prolactin (in addition to its action on the breast) maintains the activity of the corpus luteum and stimulates the secretion of progesterone (page 156)[3, 31, 3, 34]

As in the case of the testis these effects are not one sided and removal of both ovaries causes the adenohypophysis to secrete excessive quantities of FSH It has generally been held that oestrogens inhibit the production of FSH and stimulate the release of LH and prolactin while progesterone inhibits LH production and stimulates the secretion of FSH[33] These observations suggest a delicate control of ovarian activity which accords well with the accepted views concerning other trophic hormones (page 68) More recently however it has been suggested that this stimulating action upon the secretion of LH and prolactin is due not to oestrogens themselves but to oxidation products of these hormones In other words the intact oestrogen molecule depresses the secretion of FSH while its oxidation products promote the secretion of LH and prolactin An alternative view is that very low concentrations of oestrogens stimulate FSH secretion while larger doses suppress FSH and stimulate LH and prolactin secretion The truth about these issues is not known but it is possible to understand the basic relationship between the adenohypophysis and the ovary without accepting either of these theories as final

Physiology of the Menstrual Cycle

A menstrual cycle is defined as the interval which exists between the onset of one period of endometrial bleeding and the onset of the

fertilized ovum Its actions are in some instances synergistic with those of oestrogens but in other cases the two hormones are antagonistic [7] For example progesterone does not affect the endometrium unless the latter be prepared by oestrogens while in the case of uterine motility, progesterone antagonises the action of oestrogens

1) *Uterus* (a) *Endometrium* Progesterone brings the endometrium into the secretory phase if the mucosa has been previously prepared by the action of oestrogens (Fig 27) This involves thickening of the mucosa oedema of the stroma the appearance of glycogen droplets in the glandular cells together with the corkscrew form of the glands and tight coiling of the spiral arterioles In the absence of fertilization the corpus luteum regresses and the consequent withdrawal of progesterone is associated with menstrual bleeding Should fertilization occur the corpus luteum persists for about three months which is the time taken for the placenta to reach maturity The secretory condition of the endometrium enables the fertilized ovum to undergo implantation and at the same time it receives nourishment from this highly developed mucosa The chemistry of this nutrition is not understood but glutathione may be an important component in the nutritive secretion of the endometrium

(b) *Myometrium* Progesterone inhibits uterine motility which may assist in implantation of the fertilized ovum [8]

2) *Vagina* If the vagina be previously prepared by the action of oestrogens *progesterone will bring about mucification of the* mucosa Vaginal smear at this stage shows folding of the corners of the mucosal cells very few cornified cells and massive infiltration with leucocytes (Fig 30)

3) *Breast* Progesterone will only stimulate the breast if it has been prepared by the action of oestrogens It then promotes the development of the lobule alveolar pattern (page 132)

4) *Ovaries* Small doses of progesterone in the presence of oestrogens stimulate the secretion of LH which in turn promotes the development of the corpus luteum Larger doses suppress LH secretion and eventually cause ovarian atrophy by inhibiting pituitary secretion of gonadotrophins

Metabolism[29] Progesterone stimulates the catabolism of protein and increases the urinary excretion of sodium and chloride The action upon protein catabolism seems to be a direct metabolic effect but that upon salt excretion probably involves inhibition of the action of adrenocortical steroids upon the kidney

trations of these two hormones reach a certain level Ovulation is succeeded by the formation of the corpus luteum as the result of LH activity and by the secretion of progesterone due to the stimulating effect of prolactin upon the corpus luteum Meanwhile the corpus luteum reaches its maximal development about 7 days after its appearance (i e the 21st day of the cycle) and a gradual *increase in oestrogen secretion from its cells at about this time* causes the secretion of FSH which begins to prepare a group of follicles for the next cycle (Fig 25)

ENDOMETRIAL CYCLE The endometrium begins to build itself up from the basalis layer during the concluding hours of menstrual bleeding As the secretion of oestrogens rises these hormones stimulate endometrial development to the proliferative phase which reaches its maximal development at the time of ovulation Without any abrupt transition the changes characteristic of the secretory phase begin to appear as the result of progesterone *secretion by the corpus luteum Should fertilization occur the* secretory endometrium persists and receives the newly fertilized ovum

ANOVULATORY CYCLES It may happen that a Graafian follicle fails to discharge its ovum and so no corpus luteum can develop Gradually atresia of the follicle occurs and the secretion of oestrogens declines The fall in oestrogen levels in the blood may precipitate endometrial bleeding This type of cycle is normal at puberty and at the menopause and may occur from time to time during the child bearing span of life The occurrence of anovulatory cycles can be detected by the absence of those events which indicate the presence of a functional corpus luteum (page 108) The occurrence of such cycles draws attention to the importance of realising that endometrial bleeding does not necessarily indicate previous ovulation

Urinary Hormone Levels During the Menstrual Cycle

OESTROGENS Urinary oestrogens show two distinct rises in a normal cycle Of these the first occurs just prior to ovulation and is followed by a fall and later by a second rise which may exceed the first in magnitude The second rise occurs during the luteal phase but varies somewhat in the time of its appearance

GONADOTROPHINS The excretion of gonadotrophic hormones shows a distinct peak just after ovulation A second peak during the luteal phase has been described but *is not constant*

following episode of bleeding in the normal non pregnant primate It is divided into 3 phases—the menstrual or bleeding phase the follicular phase which lasts till the time of ovulation and the luteal phase which ends with the onset of bleeding The menstrual cycle is the outcome of cyclical activity at three levels —the adenohypophysial cycle the ovarian cycle and the endometrial cycle A menstrual cycle is generally regarded as starting *on the first day of menstruation (Fig 25) It should be emphasised* that the dissection of the menstrual cycle into phases and cycles though convenient from a descriptive point of view is highly artificial The events of the cycle are integrated in such a way that the so called phases move imperceptibly one into another

PITUITARY CYCLE Before the onset of menstruation the adenohypophysis begins to secrete increasing quantities of FSH *which stimulates the development of a group of primary follicles* of which one is eventually singled out to reach maturity It is now believed that the stimulus to this secretion of FSH is a low but gradually increasing concentration of oestrogen secreted by the corpus luteum By the onset of menstruation the chosen follicle for the next cycle has developed an antrum containing liquor folliculi and now prepares for the final changes which will result in rupture and the release of its ovum (Fig 25) At this point oestrogen secretion reaches levels which begin to inhibit FSH secretion and to stimulate LH production Towards the end of menstruation LH together with FSH stimulate oestrogen secretion from the *new follicle Oestrogens appear in ever increasing* quantities and the new follicle bulges from the surface of the ovary At a certain level of $\frac{FSH}{LH}$ in the blood ovulation occurs—usually about 14 days after the onset of the previous menstrual cycle (Fig 25) LH then converts what remains of the collapsed follicle into the corpus luteum and the cells which *formerly secreted oestradiol now secrete in addition progesterone*[35] The secretion of oestrogen also probably evokes the appearance of prolactin which maintains the corpus luteum and stimulates the secretion of progesterone The concentration of oestrogens *in the blood now falls but after a variable period of* time during the luteal phase it begins to rise again

OVARIAN CYCLE The ovarian cycle consists of the maturation of the follicle under the influence of FSH the secretion of oestrogen due to the synergistic action of FSH and LH followed by ovulation at a point when the relative concen

by the part played by the adrenal cortex in bringing about menstrual bleeding since there is evidence of increased cortical activity just before the onset of menstruation[38] It is generally believed that the cortex plays only a minor part in the mechanism of menstruation

Physiology of Pregnancy

Fertilization of an ovum followed by its implantation in the endometrium interrupts the reproductive cycle of woman and introduces a complicated pattern of endocrine activity Fertilization usually occurs in the fallopian tube within 24 hours of coitus It takes the fertilized ovum about 3 days to reach the uterus and another 4 to 6 days to become implanted Therefore implantation or nidation as it is sometimes called takes place on the 22nd or 23rd day after ovulation coinciding with the maximal blood levels of progesterone[39] The corpus luteum does not regress but develops further and the secretory endometrium becomes converted into the decidual mucosa The cells of the compacta acquire more cytoplasm and develop a polygonal shape which under the microscope gives the mucosa the appearance of a mosaic pattern Meanwhile the fertilized ovum develops around itself a series of projections called the chorionic villi from which the foetal placenta develops The villi consist of two distinct layers an inner cytotrophoblast composed of columns and islands of cells together with the Langhans cells and an outer mass of tissue called the syncytial trophoblast

ENDOCRINE FUNCTION OF THE PLACENTA The placenta secretes at least 3 hormones

(i) Oestrogens
(ii) Progesterone
(iii) Chorionic gonadotrophin

Oestrogens and progesterone are secreted by the syncytial trophoblast while chorionic gonadotrophin comes from the cytotrophoblast

(i) *Oestrogens* The placenta gradually usurps the ovarian role in oestrogen secretion and produces oestradiol oestrone and oestriol The excretion of oestrogens during the fertile cycle shows the expected second (luteal) rise and continues to rise steadily until about the 14th week of pregnancy when a sharper rise occurs and continues until term (Fig 31) These levels of oestrogen secretion inhibit the production of FSH and so follicle matura

PREGNANEDIOL Pregnanediol first appears in the urine just after ovulation and rises in concentration until the middle of the luteal phase and then falls until finally it is absent from the urine during menstruation

ANDROGENS Androgen excretion and urinary 17 ketosteroids do not show any consistent variation throughout the menstrual cycle

Physiology of Menstruation

Menstrual bleeding serves no useful function It represents the recognition on the part of the uterus that fertilization has failed to occur Menstrual bleeding may be defined as cyclic uterine bleeding which follows ovulation corpus luteum formation and the development of a progestational endometrium It is however *sufficient to stipulate the presence of a progestational endometrium* because in the absence of the administration of exogenous progesterone this implies ovulation and corpus luteal formation without which a secretory endometrium is not possible It should be noted however that some authors regard such a definition as serving no useful purpose and define menstrual bleeding as periodic physiological bleeding from the uterine mucosa without necessarily implying previous ovulation[36 37]

Mechanism The classical experiments of Markee have shown that the rapid withdrawal of oestrogen or progesterone or both will promote bleeding withdrawal in either case must be abrupt because a gradual fall in concentration of either hormone in the blood does not bring about haemorrhage[36 37]

The withdrawal of oestrogen like the withdrawal of progesterone causes the endometrium to shrink but the mechanism is different in each case Oestrogen withdrawal causes shrinkage of the stroma due to reabsorption of oedema fluid while progesterone withdrawal causes involution of the endometrial glands which eventually leads to narrowing of the stroma[36 37] In either case buckling of the arterioles leads to stasis of the blood necrosis of the mucosa and bleeding It can therefore be seen that ovulation and progesterone secretion are not essential for the occurrence of bleeding *There is no qualitative difference between the bleeding* of oestrogen withdrawal and that which follows corpus luteal activity It has been suggested that a local toxin of unknown composition may be the ultimate factor in precipitating the onset of bleeding but the existence of such a substance remains hypothetical[38] Certain workers on the other hand have been impressed

by the part played by the adrenal cortex in bringing about menstrual bleeding since there is evidence of increased cortical activity just before the onset of menstruation[38] It is generally believed that the cortex plays only a minor part in the mechanism of menstruation

Physiology of Pregnancy

Fertilization of an ovum followed by its implantation in the endometrium interrupts the reproductive cycle of woman and introduces a complicated pattern of endocrine activity Fertilization usually occurs in the fallopian tube within 24 hours of coitus It takes the fertilized ovum about 3 days to reach the uterus and another 4 to 6 days to become implanted Therefore implantation or nidation as it is sometimes called takes place on the 22nd or 23rd day after ovulation coinciding with the maximal blood levels of progesterone[39] The corpus luteum does not regress but develops further and the secretory endometrium becomes converted into the decidual mucosa The cells of the compacta acquire more cytoplasm and develop a polygonal shape which under the microscope gives the mucosa the appearance of a mosaic pattern Meanwhile the fertilized ovum develops around itself a series of projections called the chorionic villi from which the foetal placenta develops The villi consist of two distinct layers an inner cytotrophoblast composed of columns and islands of cells together with the Langhans cells and an outer mass of tissue called the syncytial trophoblast

ENDOCRINE FUNCTION OF THE PLACENTA The placenta secretes at least 3 hormones

(i) Oestrogens
(ii) Progesterone
(iii) Chorionic gonadotrophin

Oestrogens and progesterone are secreted by the syncytial trophoblast while chorionic gonadotrophin comes from the cytotrophoblast

(i) *Oestrogens* The placenta gradually usurps the ovarian role in oestrogen secretion and produces oestradiol oestrone and oestriol The excretion of oestrogens during the fertile cycle shows the expected second (luteal) rise and continues to rise steadily until about the 14th week of pregnancy when a sharper rise occurs and continues until term (Fig 31) These levels of oestrogen secretion inhibit the production of FSH and so follicle matura

tion is suspended and the ovary shows histological evidence of regression[40]

(ii) *Progesterone* Progesterone secretion may be studied indirectly by means of the urinary concentration of pregnanediol or the pregnanediol complex (page 115), which during pregnancy represents the sum of corpus luteal and placental progesterone Pregnanediol excretion does not show the expected premenstrual fall, but continues to rise steadily and later more rapidly parallel with oestrogen excretion until at term levels between 50 and 100 mgm per 24 hours appear (Fig 31)

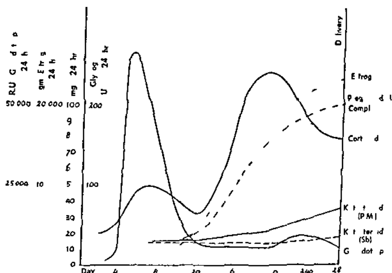


Fig 31 The urinary excretion of hormones during pregnancy (*Venning Normal and Pathological Physiology of Pregnancy Courtesy of Williams and Wilkins*)

(iii) *Chorionic Gonadotrophin* The placenta secretes a hormone peculiar to itself and to certain neoplasms This hormone is called chorionic gonadotrophin and resembles pituitary luteinizing hormone in its action Chorionic gonadotrophin is *secreted by the cytotrophoblast of the chorionic villi and is* excreted in the urine almost immediately following implantation with the result that its presence in urine may be detected before the first missed menstrual period It is detection of this hormone in urine which forms the basis of certain pregnancy tests The presence of chorionic gonadotrophin in the urine means no more than that active chorio placental tissue is present in the body the foetus may be alive or dead and indeed certain tumours produce the same

hormone About 20 days after implantation the concentration of chorionic gonadotrophin in the urine reaches a peak after which it falls to a lower level which it maintains until term

Chorionic gonadotrophin stimulates the corpus luteum of pregnancy and thereby promotes the secretion of oestrogens and progesterone from the ovary during the early months of pregnancy The hormone is capable of producing decidual changes in the endometrium and can evoke the endocrine changes of pregnancy in the absence of a fertilized ovum (pseudopregnancy)

There is some evidence to show that chorionic gonadotrophin also stimulates the syncytial trophoblast to secrete oestrogen and progesterone after the corpus luteum of pregnancy has regressed There seems litt e doubt that the hormone must play an important part in the physiology of pregnancy but the exact extent of its role remains somewhat uncertain

Other Hormones During pregnancy the secretion of pituitary gonadotrophins appears to be suppressed [9] while ACTH appears in the blood in increasing quantities after the third month It has been suggested, as the result of experimental studies of placental function that at least some of this increase in ACTH secretion may come from the placenta but this suggestion is open to doubt

Pregnancy Tests A number of tests have been devised to facilitate the diagnosis of pregnancy at a time before this is possible by clinical examination of the patient Most of the tests in current use depend upon the detection of chorionic gonadotrophin in the urine in concentrations which exceed those of pituitary gonadotrophin in non pregnant women A biological end point is therefore selected which will distinguish the urinary levels of chorionic gonadotrophin encountered in pregnancy from those of pituitary gonadotrophins in the non pregnant state Such a distinction is possible at the time of implantation (generally the twenty fifth day of the cycle) that is just before the first missed period The accuracy of these tests is slightly greater about two weeks after implantation

The classical Aschheim Zondek test made use of the appearance of haemorrhagic follicles in the ovaries of immature mice (or in a later modification rats) The test was accurate but required 96 hours before giving a result

The Friedman test needs 48 hours and makes use of the adult female rabbit which has been isolated from the male for at least 3 weeks The presence of microscopic haemorrhagic ovarian follicles indicates a positive test These two tests are accurate but

involve the sacrifice of one or two animals for each examination

A number of accurate tests using frogs and toads of various species have been devised These tests possess two overwhelming advantages namely the results can be reported in two hours and each animal can be used repeatedly Among these methods the Xenopus toad test is now widely used, as is the Galli Mainini test, which uses the male South American toad Tests using male animals are positive if the urine contains evidence of seminal dis charge, whereas in female animals the extrusion of ova indicates a positive result

Tests indicating the presence of chorionic gonadotrophin are positive not only in cases of pregnancy but also in patients of either sex suffering from neoplasms which secrete this hormone

One chemical test of pregnancy has been devised to measure the increased urinary excretion of pregnanediol in pregnancy This is called the Guterman test and involves a colorimetric assay

Parturition

The mechanism which precipitates parturition is obscure It has been suggested that oxytocin (page 169) may play an important role or that uterine distension and high blood levels of oestrogens and progesterone may be factors So far, none of these changes has been shown to be essential to the onset of labour

Sperm Transport

The inherent motility which the spermatozoa acquire when they are suspended in seminal fluid is largely responsible for their reaching the fallopian tubes However muscular contractions of the female genital tract may assist in sperm transport and the less viscous alkaline cervical mucus produced under the influence of oestrogens appears to encourage the entry of spermatozoa into the uterus

REFERENCES

1 Novak E. Gynaecological and Obstetrical Pathology W B Saunders Philadelphia 1947
2 Bailey's Textbook of Histology Williams & Wilkins Baltimore 1948
3 Daron G H Am J Anat 58 349 1936
4 Kundrat H & Engelmann G J cited in Contrib Embryol 21 141 1933
5 Markee J E Contrib Embryol 28 219 1940
6 Novak E & Tehinde R W J A M A 83 900 1924
7 Reynolds S R M J A M A 135 552 1947

8 Papanicolaou G N Am J Anat. 52 519 1933
9 De Allende I L.C. et al Contrib Embryol 31 1 1943
10 Corner G W Phys Rev 19 154 1938
11 Claesson L & Hillarp N.A Acta Physiol Scandinav 13 115 1941 & 14 102 1947
12 Emmens C.W J Endocrinol 3 174 1942
13 Biskind M.S & Biskind C R Endocrinology 31 108 1942
14 Biskind M S & Biskind C R Science 94 462 1941
15 Cantarow E et al Science 101 558 1945
16 Szego C M & Roberts S Proc Soc Exp Biol & Med. 61 161 1946
17 Fishman W H & Fishman L W J Biol Chem 152 487 1944
18 Perlman W H The Hormones Academic Press N Y Vol 1 1948
19 Kober S Biochem Ltscher 239 98 1938
20 Allen E. et al Am J Anat. 34 13 1924
21 Smith G van S et al Am J Physiol 121 98 1938
22 Albright F & Reifenstein E.C Jr Parathyroid Glands & Metabolic Bone Disease Williams & Wilkins Co Baltimore 1948
23 Allen E Glandular Physiology and Therapy Chicago 1942
24 Pincus G The Hormones Academic Press N Y 2 1950
25 Bloch K J Biol Chem 157 761 1945
26 Marrian G F Recent Progress in Hormone Research 4 43 1949
27 Courrier R Vitamins & Hormones 8 179 1950
28 Corner G W Glandular Physiology & Therapy A M A Chicago 1942
29 Gaunt R. & Hays H W Science 88 576 1938
30 Frank, R T J A M.A 114 1504 1940
31 Coppedge R.L & Segaloff A J Clin Endocrinol 11 465 1951
32 McArthur J W The Endocrine Society 34th Meeting 1952
33 Smith G V & Smith O W New England J Med 215 908 1936
34 Smith G van S Textbook of Endocrinology Saunders Philadelphia 1950
35 Astwood E B Endocrinology 28 309 1941
36 Markee J E Contrib Embryol 28 219 1940
37 Markee J E Bull N Y Acad Med 24 253 1948
38 Smith O W Menstruation and Its Disorders p 187 Charles C Thomas Springfield 1950
39 Hertig A.T and Rock J Am J Obst and Gynaec 47 149 1944
40 Rakoff A E Am J Obst and Gynaec 38 371 1939

involve the sacrifice of one or two animals for each examination

A number of accurate tests using frogs and toads of various species have been devised These tests possess two overwhelming advantages namely the results can be reported in two hours and each animal can be used repeatedly Among these methods the Xenopus toad test is now widely used as is the Galli Mainini test, which uses the male South American toad Tests using male animals are positive if the urine contains evidence of seminal dis charge whereas in female animals the extrusion of ova indicates a positive result

Tests indicating the presence of chorionic gonadotrophin are positive not only in cases of pregnancy but also in patients of either sex suffering from neoplasms which secrete this hormone

One chemical test of pregnancy has been devised to measure the increased urinary excretion of pregnanediol in pregnancy This is called the Guterman test and involves a colorimetric assay

Parturition

The mechanism which precipitates parturition is obscure It has been suggested that oxytocin (page 169) may play an important role or that uterine distension and high blood levels of oestrogens and progesterone may be factors So far none of these changes has been shown to be essential to the onset of labour

Sperm Transport

The inherent motility which the spermatozoa acquire when they are suspended in seminal fluid is largely responsible for their reaching the fallopian tubes However muscular contractions of the female genital tract may assist in sperm transport and the less viscous alkaline cervical mucus produced under the influence of oestrogens appears to encourage the entry of spermatozoa into the uterus

REFERENCES

1 Novak E Gynaecological and Obstetrical Pathology W B Saunders Philadelphia 1947
2 Bailey s Textbook of Histology *Williams & Wilkins Baltimore 1948*
3 Daron G H Am J Anat 58 349 1936
4 Kundrat H & Engelmann G J cited in Contrib Embryol 21 141 1933
5 Markee J E Contrib Embryol 28 219 1940
6 Novak E & Telinde R W J A M A 83 900 1924
7 Reynolds S R.M J A M.A 135 552 1947

Embryology

It is surprising that an organ which does not function until so late in life and one which appears so recently in evolution should develop so early in embryonic life At the beginning of the sixth week an ectodermal thickening occurs as a longitudinal band between the bases of the limb buds (Fig 32) This structure is called the milk line and in man is only prominent in the pectoral region Each breast begins as a thickening and downgrowth from the milk line During the fifth month between 15 and 25 solid cords bud off to form the primary milk ducts where these ducts open onto the surface the epidermis becomes elevated to form the nipple (Fig 33)

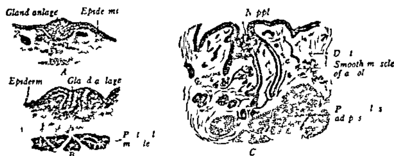


Fig 33 The development of the human mammary gland as illustrated by vertical section — (A) at two months (B) at four months (C) at seven months (*Arey*)

Histology

Each mammary gland consists of about 20 closely adjoining lobes radiating from the nipple Each lobe possesses its own duct which opens independently at the nipple These lactiferous ducts as they are called each show a dilation below the nipple (the lactiferous sinus) The lactiferous duct divides into smaller and smaller branches the smallest of these are the alveolar ducts which end in blind dilatations from which spherical sacs called alveoli will develop In this way each lobe becomes divided into a number of units called lobules in accordance with the sub-divisions of the lactiferous ducts Each lobe is therefore an independent compound alveolar gland with its own duct It should be noted that the terms alveoli and acini are used synonymously

Throughout the breast a network of contractile myoepithelial tissue occurs over the stromal surface of the alveoli (Fig 34) This tissue can be demonstrated by means of silver stains Reflex

CHAPTER VI

THE BREAST

Introduction

The presence of mammary glands is the distinctive feature of mammalian animals and the function of these glands is to secrete milk from certain precursors furnished by the blood The processes by which milk is synthesised and expelled from the mammary gland together constitute the function of lactation which normally provides the sole source of nourishment for the newborn mammal The breast is not an endocrine gland but its function is so intimately associated with the secretions of the endocrine glands that it is appropriate to summarise these associations

Evolution

The initial stages in the evolution of the milk secreting glands are lost in antiquity The individual tubes of the mammary glands of such primitive forms as the platypus closely resemble sweat glands This has lead some workers to suggest that the breast has developed from modified sweat glands[1 18]

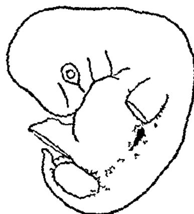


Fig 32 The milk line represented diagrammatically in a human embryo of six weeks The area which gives rise to the adult breast is shown in black (Arey)

fibrous and fatty stroma At the same time the lactiferous ducts become elongated and undergo complex branching The lining cells increase in number and epithelial buds appear at the ends of the alveolar ducts In this way each lobe comes to contain a large number of lobules but alveoli are not yet seen With the onset of regular menstrual periods associated with ovulation the breast undergoes cyclical activity in time with the events of the ovarian cycle Two phases are generally described—proliferative and regressive (Fig 35 (a))

Proliferative phase This phase begins during the second week of the cycle (i e about 10 days after the onset of menstruation) and continues until a few days before the next period The breast enlarges and becomes finely nodular to palpation The ducts dilate and contain a little secretion while the connective tissue around the ducts becomes oedematous Lobules develop and expand acquiring some alveoli This is the first appearance in the breast *of alveoli (Fig 35 (a)) which develop from the dilated ends of* the alveolar ducts

Regressive phase This phase involves a decline in the lobular development of the previous phase Alveoli disappear and the alveolar ducts regress epithelial cells shrink and some desquamate The stroma shows infiltration by round cells

PREGNANCY The changes seen in the breast during pregnancy are an extension of those seen during the phase of proliferation The emphasis is upon continued branching of the ducts with widespread development of alveoli The alveoli become lined by cuboidal epithelium and contain an increasing quantity of secretion (Fig 35 (b))

LACTATION Soon after birth the lobules and their ducts take up *their definitive functions—the secretion* and storage of milk Secretion occurs from the cells lining the dilated alveoli and the duct system expands to act as a reservoir for newly formed milk After the conclusion of lactation the breast slowly returns to the non pregnant state undergoing the cyclical changes already described

CLIMACTERIC After the climacteric the breast shows irregular regressive changes Alveoli and lobules disappear the lactiferous ducts shrink and the stroma increases in density Some what later cystic dilation of the larger ducts with sclerosis and obliteration of their smaller branches brings the breast to its senile form

contraction of these myoepithelial cells produces ejection of milk from the smaller ducts and alveoli into the larger ducts and lactiferous sinuses This description of breast histology applies to the condition of the organ at the end of puberty Thereafter it undergoes certain changes according to the demands placed upon it *On the other hand the breast of the newborn shows some features of interest*



Fig 34 Microscopic section of the breast showing part of the surface of a contracted alveolus and a myoepithelial cell with nucleus (n) and branching processes (*A C Richardson 1949*)

THE NEONATAL BREAST During the first few weeks of extrauterine life the breast shows signs of activity in the form of dilation of the ducts which become lined by two layers of epithelial cells together with a considerable vascular development In about 60 per cent of the newborn glandular tissue is palpable behind the nipples After about three weeks this tissue can no longer be felt and the gland enters a period of quiescence until puberty

ADOLESCENCE The first sign of puberty in girls is an enlargement of the areolae which become pigmented The breast soon increases in size chiefly owing to the development of a

The effect of oestrogens on the human breast has been studied in men and women but more detailed experiments are needed to confirm the findings of earlier workers Among animals three types of response to oestrogens are found (i) in the mouse, rat and rabbit physiological doses produce duct development, but not alveolar growth (ii) in the monkey guinea pig cow and goat ex-

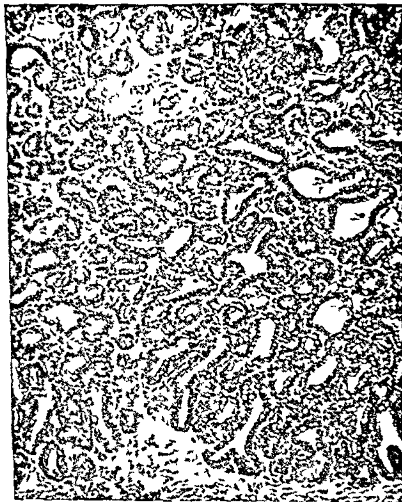


Fig 35 (*b*) The human breast during the eighth month of pregnancy showing well developed acini

THE ACTION OF HORMONES ON THE BREAST

OESTROGENS Oestrogens cause an increase in length of the lactiferous ducts together with the formation of new branches and an increase in the size of the lining cells At the same time the dilated ends of the terminal branches of the duct system become more prominent Outside the duct system the fibrous and fatty stroma proliferates Oestrogens also cause an increase in the size of the areola and nipple together with some pigmentation of these structures[2 3 4 5 6]



Fig 35 (a) The human breast during the regressive phase showing dilated ducts lined by well developed epithelium

MAMMOGENIC HORMONES[1, 13, 14] In order that oestrogens and progesterone may exert their characteristic effects upon breast tissue the adenohypophysis must be capable of normal function[11] However local application of oestrogens to the skin over one breast will cause that breast alone to develop but this local action does not occur in hypophysectomised animals Some workers have extended these observations and conclude that the adenohypophysis secretes two hormones called mammogen I and mammogen II[12, 13, 14] The former is secreted under the influence of oestrogens and promotes duct development while mammogen II is secreted in response to progesterone and causes alveolar development In other words these workers believe that the effects of oestrogens and progesterone upon the breasts are mediated through the pituitary gland No final decision can be reached about this issue at present but the existence of two additional pituitary hormones should be regarded with some scepticism

Indirect evidence against the existence of mammogenic hormones has emerged from experiments on hypophysectomised rats In such rats it has been shown that complete mammary development can be attained by the combined use of oestrogens progesterone prolactin ACTH and growth hormone It would appear that ACTH is necessary to restore the animal to a more nearly normal physiological state but that growth hormone has a specific role in mammary development Appropriate changes in the relative doses of the five hormones mentioned will produce lactation Prolactin can under certain conditions be replaced by placental extracts These observations make it unnecessary to postulate the existence of mammogenic hormones

Further experiments with hypophysectomised rats have shown that although oestrogens and progesterone do not stimulate mammary development in the absence of the adenohypophysis the capacity to respond to these hormones is restored by the administration of insulin This observation accords well with the fact that growth hormone and under certain conditions prolactin can stimulate the secretion of insulin from the pancreatic islets[8]

CHORIONIC GONADOTROPHIN[15] So far as is known chorionic gonadotrophin affects the breasts only indirectly by acting upon the ovary and the placenta

ENDOCRINE CONTROL OF THE BREAST

As with other aspects of endocrine function it is impossible to state the hormonal control of the breast in its final form Experimental evidence derived from mammals other than man requires

tensive alveolar development occurs and (iii) in the dog oestrogens alone produce very little response

PROGESTERONE Progesterone requires the previous action of oestrogens before it can stimulate the breast The characteristic effect of the hormone is to promote the development of alveoli In woman alveoli do not appear until progesterone acts upon the breast which has been prepared by oestrogens

PROLACTIN Prolactin exerts three effects upon the breast[8]

1) It acts indirectly by stimulating the ovarian production of progesterone (page 156)

2) It is partly responsible for the rapid development of the breast during the later stages of pregnancy by direct action upon the gland

3) It stimulates the secretion of milk

OXYTOCIN Oxytocin stimulates the myoepithelial cells of the breast together with smooth muscle in the walls of the lactiferous ducts and thereby causes milk ejection

GROWTH HORMONE Recent studies have suggested that growth hormone is partly responsible for the maintenance of lactation once this has been initiated[3]

ANDROGENS[4 9 10] In experimental animals, androgens cause striking stimulation of the breast tissue in both sexes These hormones cause the development of alveoli which is interesting in view of the chemical resemblance between progesterone and certain androgens The significance of these findings in terms of normal function is unknown

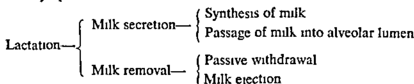
ADRENAL STEROIDS[4 7 11] Deoxycorticosterone promotes alveolar development and it is known that the cortex is essential for lactation Detailed studies of the action of glucocorticoids on the breast have only recently been undertaken

In some experiments hydrocortisone appears to inhibit breast development while in others the hormone stimulates the growth of mammary tissue and also promotes lactation Selye believes that the stimulating effect of glucocorticoids upon the breast accounts for the occurrence of breast development and lactation after exposure to acute stress For example this phenomenon has been reported after extensive skin burns

THYROID HORMONE Thyroid hormone appears to assist the process of lactation but the role of the gland in the normal function of the breast is uncertain

called the double threshold theory of Folley and Malpress This states that low concentrations of oestrogens stimulate the lactogenic function of the adenohypophysis while higher concentrations inhibit lactation Lactogenic levels of oestrogen may be rendered inhibitory by an appropriate concentration of progesterone At parturition a fall in the ratio of progesterone to oestrogen removes this inhibitory effect which leaves the lactogenic action of oestrogens unopposed[19] These experiments cannot be applied to man with any confidence at this stage and it remains to define these high and low concentrations of oestrogens with more precision

The process of lactation involves the two separate functions of milk secretion and milk removal Milk secretion itself involves two processes namely the synthesis of milk by the cells of the alveolar epithelium and the passage of the milk so formed from the cytoplasm of these cells into the alveolar lumen



MILK REMOVAL In the lactating mammary gland the greater portion of the milk secreted by the alveolar cells remains within the alveolar lumen and smaller ducts A smaller portion passes into the larger ducts and sinuses this portion can be removed at once by suckling or milking and its removal is not dependent upon the contractile mechanism of the gland—the removal of this portion of the milk is referred to as passive withdrawal The greater portion of the milk requires active ejection from the alveoli and fine ducts into the larger ducts and sinuses before it is available to the suckling young or the artificial milker This process of milk ejection involves reflex contraction of the myoepithelium which forms a network over the stromal surface of the alveoli (Fig 34)

The myoepithelial or basket cells are flattened stellate cells lying upon the outside of the alveoli between the epithelium and the basement membrane with their processes enveloping the alveolar surface Living mammary tissue has been shown under the microscope to respond to topically applied oxytocin by contraction of the basket cells which can be seen to cause contraction of the alveoli forcing milk into the larger ducts

The act of suckling has been shown to stimulate not only milk ejection but also the secretion of milk Folley has recently produced good evidence for believing that in animals oxytocin not

cautious interpretation because the physiology of the breast shows important differences between one species and another However, it is possible to outline the effect of hormones upon the various phases of breast activity At birth the breast is only partly developed and consists of blind branching tubules incapable of secreting milk However soon after birth histological evidence of stimulation is apparent and some form of secretion may be seen These effects are thought to result from the action of maternal oestrogens which have crossed the placenta Withdrawal of these maternal oestrogens together with the presence of prolactin in neonatal blood may be responsible for the production of witches milk, as the secretion of the neonatal breast is called[16] The awakening of breast activity at puberty is generally held to be the result of oestrogen stimulation The appearance of alveoli and the phase of breast proliferation which occurs during the fully established menstrual cycles is due to the synergistic action of oestrogens and progesterone Whether these hormones act directly upon the glandular tissue of the breast or evoke the secretion of pituitary hormones is uncertain The full development of lobules with their alveoli occurs during pregnancy due to the action of prolactin supported by progesterone and oestrogens

THE PHYSIOLOGY OF LACTATION

Lactation is a complex function the details of which are not wholly understood No doubt it requires the presence of prolactin and the fully developed alveolar pattern which appears during pregnancy In addition some adrenocortical hormone(s) are essential for the production of milk while growth hormone supervises the maintenance of this secretion Thyroid hormone assists the secretion of milk but is apparently not essential for this function The initiation of lactation depends in part upon the presence of prolactin in suitable concentrations in part upon the withdrawal of oestrogens (and perhaps progesterone) at parturition and finally upon nervous reflexes initiated by suckling (page 157)

Experimental studies of the factors involved in the onset of lactation have recently turned to the problem of why lactation does not occur during pregnancy This has been explained by two theories Meites believes that the high concentrations of oestrogens and progesterone during pregnancy stimulate breast growth and make the mammary tissue refractory to the action of prolactin At the same time the concentration of prolactin in the blood is *relatively* low during pregnancy The alternative explanation is

1 Maximow A.A., and Bloom W., A Textbook of Histology Saunders Philadelphia, 1943
2 Geschickter C.F., Diseases of the Breast. Lippincott Philadelphia 1943
3 Lewis D and Geschickter C.F., Ann. Surg. 100 779 1934
4 Folley S.J., Marshall s Physiology of Reproduction. London 1952
5 Gomez, E.T and Turner C.W., Proc Soc. Exper Biol N Y 34 320 1936
6 Gomez, E.T and Turner C.W., Res. Bull. Mo Agric Exp Sta. No 259 1936
7 Nelson, W O., et al. Endocrinology 33 325 1943
8 Folley S.J., Recent Progress in Hormone Research 7 107 1952.
9 Astwood E.B Geschickter C.F and Rausch E.O., Am. J Anat 61 373 1937
10 Van Wagenen, G and Folley S.J J Endocrinol 1 367 1939
11 Trentin J.J and Turner C.W., Endocrinology 29 984 1941 and 41 127 1947
12 Lyons, W R., Proc Soc. Exper Biol & Med. 51 308 1942
13 Gardner W U and White A., Proc. Soc Exper Biol & Med 48 590 1941
14 Nelson W O Ciba Colloquia on Endocrinology 4 402 1952.
15 Maddock, W O and Nelson W O J Clin. Endocrinol. 12 985 1952
16 Lyons W R., Proc. Soc Exper Biol & Med. 35 645 1937
17 Cowie A T and Folley S.J., The Neurohypophysis p 183 Butterworth, London, 1957
18 Turner C.W., The Comparative Anatomy of the Mammary Gland Columbia Mi. 1939
19 Tindal J.S and McNaught, M L. Modern Trends in Endocrinology Butterworth, 1958

only stimulates the myoepithelium of the breast in response to the stimulus of suckling but at the same time promotes the release of prolactin from the adenohypophysis[17] In this way oxytocin may serve to integrate the two aspects of lactation by promoting the replacement of the milk which it causes to be ejected

In the hope of gaining a better understanding of the endocrine factors responsible for the maintenance of established lactation recent studies of galactopoiesis (the stimulation of established lactation) have been undertaken These investigations have shown that the galactopoietic action of growth hormone is associated with a small but definite rise in respiratory quotient oxygen uptake and utilization of glucose by slices of lactating mammary tissue Thyroxine shows well marked galactopoietic activity while the naturally occurring corticosteroids show little effect upon established lactation Surprisingly prolactin shows very little galactopoietic action although it causes considerable stimulation of lactation in its early stages before this function is fully established Prolactin has given disappointing results when used to stimulate established but inadequate lactation in parturient women However prolactin together with ACTH produces a sharp rise in the activity of coenzyme A in lactating mammary glands in vitro suggesting that the two hormones are synergistic in their action upon lactation

THE MALE BREAST[2]

Until the period of neonatal activity the male breast closely resembles the female Thereafter it undergoes a more complete regression than the female gland At puberty about 70 per cent of boys show a palpable button shaped node beneath the nipple Microscopically this tissue shows duct stimulation which resembles the changes seen in the female gland but on a smaller scale Between the ages of 16 and 17 involutional changes occur, with collapse of tubules and condensation of connective tissue The male breast has now run its full life span and gradually sclerosis of the mammary stroma together with obliteration of the ducts and blood vessels sets in

REFERENCES

An excellent review of the literature on the endocrine aspects of the breast is found in Marshall s Physiology of Reproduction" (London 1952) Geschickter s Diseases of the Breast (Lippincott Philadelphia 1943) gives a detailed description of the normal breast while Folley and Malpress have reviewed the whole subject exhaustively in 'The Hormones (Academic Press N Y Vol 1 1948)

The term adenohypophysis refers to what was once called the anterior pituitary gland together with the pars intermedia The term pars tuberalis refers to that portion of the adenohypophysis which spreads over the base of the brain around the pituitary stalk The term neurohypophysis is applied to what was once called the posterior pituitary gland

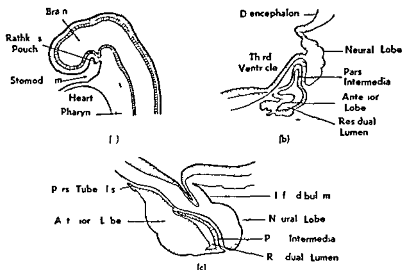


Fig 37 (*a*) Diagrammatic sect on through an embryo of four weeks to indicate the origin of Rathke s pouch
(*b*) Midsagittal section at eight weeks to show the development of the pituitary gland
(*c*) Midsagittal section at eleven weeks (*Arey*)

Embryology

The pituitary gland has a double origin partly from epithelium and partly from the brain wall The adenohypophysis is derived from an ectodermal pocket which arises from the posterior wall of the nasopharynx This sac known as Rathke s pouch (Fig 37) is already distinct in embryos of 4 weeks It elongates and soon comes in contact with a sac like extension of the infundibulum which is the forerunner of the neurohypophysis Rathke s pouch at first flat extends laterally and caudally growing around the neurohypophysis and loses its connection with the nasopharynx at the end of the second month (Fig 37) The original cavity of the pouch becomes almost obliterated

CHAPTER VII

THE ADENOHYPOPHYSIS

Introduction

The adenohypophysis exerts such an important influence upon other endocrine structures that it is generally looked upon as the master gland It is lodged within the sella turcica of the sphenoid bone surrounded by the dura mater and attached to the floor of the third ventricle by a thin stalk here it lies in close relationship with the optic chiasma Throughout this book the nomenclature of Rioch Wislocki and O Leary[1] 1940 (Fig 36), is used to designate the various parts of the gland

ADENOHYPOPHYSIS
1 Pars distalis
2 Pars tuberalis
3 Pars intermedia

NEUROHYPOPHYSIS
1 Infundibular process
2 Infundibular stem
3 Median eminence of the tuber cinereum

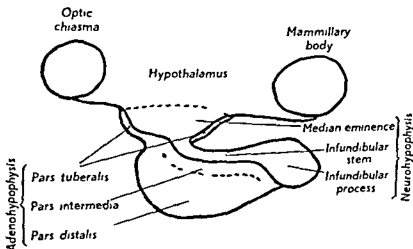


Fig 36 Diagram of a sagittal section of the pituitary gland to illustrate the terminology of Rioch Wislocki and O Leary (*G W Harris*)

(II) *Red acidophil cells* These cells have larger granules than the orange acidophils and predominate in the centre of the pos terior portion of the gland They frequently occur in clusters of 8 to 10

(III) *Blue basophil cells* Larger than either type of acidophil the blue basophil contains cytoplasmic granules which show the histochemical behaviour of glycoprotein Apart from these fine granules the cells contain vesicles which are filled with glyco protein and probably result from fusion of granules

(IV) *Purple basophil cells* This cell type resembles the blue basophil but the granules are not densely packed and do not form vesicles They are most frequent in the centre of the gland anter-iorly especially near the larger blood vessels

(V) *Pale basophil cells* Pale basophils are large cells and pos sess large nuclei Granules appear along the cell margins and near the Golgi body Elsewhere the cytoplasm has a characteristic foamy appearance

(VI) *Chromophobes* This type of cell is the smallest of all and occurs in clusters

In addition an extraordinary phenomenon seen among the cells of the pars distalis referred to as cupping deserves mention this is most frequently seen between orange acidophils and purple basophils the orange cell is seen to be surrounded by the cyto plasm of the basophil in such a way that the former appears to be almost engulfed by the latter The significance of cupping is not known

Although this description of pituitary histology is based upon experimental studies using the hypophyses of dogs it is believed that similar observations apply to the adenohypophysis of man The colours used to designate the cell types are those produced by a modification of Mallory s trichrome stain preceded by nuclear staining with haematoxylin The periodic acid Schiff stain confirms the glycoprotein nature of the basophil granules

In general acidophil cells are seen posteriorly in each lateral half of the pars distalis while the chromophobes and basophils are more commonly found near the midline and in the anterior part of the gland In the individual cords of cells adjacent to the blood sinusoids chromophobes are more frequent in the centre and chromophils next to the sinusoids

A number of factors affect the appearance and the relative proportions of the cells in the adenohypophysis Basophils are more common in men acidophils in women Chromophobe cells

During the third and fourth months, the pituitary becomes more elaborate in its structure and finally assumes its definitive organisation The anterior wall of Rathke s pouch grows thick and gives rise to the cords of cells which characterise the pars distalis The posterior wall remains thin and constitutes the pars intermedia (Fig 37) Finally part of the original pouch extends forward along the infundibulum to become the pars tuberalis it develops from the fusion of paired lateral lobes which bud off from the pouch close to its original stalked connection with the nasopharynx The early neurohypophysis is transformed into a solid mass of neuroglial tissue it remains connected with the diencephalon by a permanent infundibular stalk Only the adenohypophysis will be considered in the present chapter

Histology

The adenohypophysis is made up of groups and columns of epithelial cells which are supported in a delicate reticular connective tissue Between these columns are dilated sinusoids lined by macrophages About half of the epithelial cells contain pigment granules and are therefore called chromophil cells Of these cells some show acidophil granules (alpha cells) while the remainder are characterised by basophil granules (beta cells) Alpha cells exist in numbers about three and a half times greater than beta cells Chromophobe cells are generally regarded as precursors of the chromophil cells and are especially numerous near the stalk

In recent years it has been shown that the two cell types called acidophils and basophils are not homogeneous groups Purves and Griesbach[13] have recognised two types of acidophils and three types of basophils in the adenohypophysis of the dog Characteristic of acidophil cells is the presence of cytoplasmic granules which are composed of protein and are insoluble in aqueous buffer solutions in the pH range of 4 0 to 8 0 By contrast the special feature common to basophil cells is the presence of cytoplasmic granules which are composed of glycoproteins such granules are soluble in aqueous buffer solutions The same workers describe the following cell types

(I) *Orange acidophil cells* These are the most numerous and smallest of the chromophil cells they are found throughout the gland but are especially prominent in the antero lateral parts of the pars distalis where they occur in symmetrical sharply defined zones The cytoplasm of these cells is densely packed with granules and the nucleus of each is prominent

(II) *Red acidophil cells* These cells have larger granules than the orange acidophils and predominate in the centre of the posterior portion of the gland They frequently occur in clusters of 8 to 10

(III) *Blue basophil cells* Larger than either type of acidophil the blue basophil contains cytoplasmic granules which show the histochemical behaviour of glycoprotein Apart from these fine granules the cells contain vesicles which are filled with glycoprotein and probably result from fusion of granules

(IV) *Purple basophil cells* This cell type resembles the blue basophil but the granules are not densely packed and do not form vesicles They are most frequent in the centre of the gland anteriorly especially near the larger blood vessels

(V) *Pale basophil cells* Pale basophils are large cells and possess large nuclei Granules appear along the cell margins and near the Golgi body Elsewhere the cytoplasm has a characteristic foamy appearance

(VI) *Chromophobes* This type of cell is the smallest of all and occurs in clusters

In addition an extraordinary phenomenon seen among the cells of the pars distalis referred to as cupping deserves mention this is most frequently seen between orange acidophils and purple basophils the orange cell is seen to be surrounded by the cytoplasm of the basophil in such a way that the former appears to be almost engulfed by the latter The significance of cupping is not known

Although this description of pituitary histology is based upon experimental studies using the hypophyses of dogs it is believed that similar observations apply to the adenohypophysis of man The colours used to designate the cell types are those produced by a modification of Mallory s trichrome stain preceded by nuclear staining with haematoxylin The periodic acid Schiff stain confirms the glycoprotein nature of the basophil granules

In general acidophil cells are seen posteriorly in each lateral half of the pars distalis while the chromophobes and basophils are more commonly found near the midline and in the anterior part of the gland In the individual cords of cells adjacent to the blood sinusoids chromophobes are more frequent in the centre and chromophils next to the sinusoids

A number of factors affect the appearance and the relative proportions of the cells in the adenohypophysis Basophils are more common in men acidophils in women Chromophobe cells

become increasingly prevalent with age while pregnancy brings about changes in certain of the pituitary cells which develop into the so-called pregnancy cells the origin of these cells is uncertain Castration causes an increase in the size and number of basophils these enlarged basophils come to contain a colloid like substance which pushes the nucleus to one side, producing the signet ring appearance of the so-called castration cell

Function

The only known function of the adenohypophysis is the secretion of hormones The number of these hormones is as yet uncertain but seven can be accepted as true hormones

1 Growth hormone (somatotrophin)
2 Thyroid stimulating hormone (thyrotrophic hormone)
3 Adrenocorticotrophic hormone (corticotrophin)
4 Follicle stimulating hormone
5 Luteinizing hormone (interstitial cell stimulating hormone)
6 Prolactin
7 Melanocyte stimulating hormone

Diabetogenic pancreatotrophic ketogenic and other effects have been described following the injection of pituitary extracts but between such effects and the isolation of a pure hormone a great difference exists Only the above seven hormones can be accepted at the present time as products of adenohypophysial secretion

The term gonadotrophic hormones or gonadotrophins is applied to follicle stimulating hormone and luteinizing hormone since these two hormones stimulate the gonads in both sexes The term will be used in this way throughout this book Some authors however use the word gonadotrophin to include prolactin which is logical in the female where the hormone is known to stimulate the corpus luteum of the ovary but is perhaps less precise in the male where the same word applies only to two hormones since the significance of prolactin in males is uncertain

The first accounts of pituitary function were based upon the observation of animals subjected to the operation of hypophysectomy This operation does not cause immediate death provided that brain damage is reduced to a minimum The following changes are seen as the result of hypophysectomy in animals —

1 In young animals the rate of growth is very greatly diminished Such animals remain much smaller than their normal litter

mates In adult animals loss of body protein occurs this loss is particularly evident in the skin and the skeletal muscles

2 A marked atrophy of the thyroid gland is seen and evidence of inadequate thyroid activity appears

3 The adrenal cortex decreases in size (especially its fasciculate and reticular zones) Hypophysectomised animals become very susceptible to all forms of stress and to hypoglycaemia Changes in electrolyte metabolism are not gross which suggest that mineralocorticoid secretion is unaffected by hypophysectomy The operation also produces amelioration of the diabetes which follows pancreatectomy This was first demonstrated by Houssay and such a hypophysectomised pancreatectomised animal is called a Houssay animal This amelioration of pancreatic diabetes has been attributed to the absence of growth hormone but it is possible that loss of ACTH may play some part in this phenomenon since adrenalectomy will also alleviate the diabetes of animals subjected to pancreatectomy

4 In either sex the gonads atrophy and the secondary sexual characteristics regress (or in the young they fail to develop)

5 In certain species (fish amphibians and reptiles) there is a bleaching of the pigment cells (chromatophores) of the skin and a failure of the adaptation to environmental changes normally shown by these cells

These changes (except the last) are seen in man when removal or destruction of the adenohypophysis occurs Occasionally a one sided failure of adenohypophysial function is seen in man notably failure of gonadotrophic hormone production

Some of these changes can be attributed to loss of the influence which the adenohypophysis normally exerts upon other endocrine glands (adrenal cortex thyroid and gonads) This influence is described as a trophic effect and the hormones responsible for this effect are called trophic hormones (corticotrophin thyrotrophic hormone gonadotrophins) The suffix trophic is to be preferred to tropic since the former conveys the nature of this relationship between the adenohypophysis and the other endocrine glands more exactly Trophic (Greek trophe nutrition) refers to the nourishing effect of the adenohypophysis tropic (Greek tropos turning) is intended to indicate a turning towards

It is apparent therefore that the adenohypophysis exerts a widespread physiological influence which gives it a cardinal position among the endocrine glands

1 GROWTH HORMONE

CHEMISTRY Growth hormone has been obtained in crystal line form It is a complex protein with a molecular weight of about 44 000 and contains 16 per cent of nitrogen and 1 3 per cent sulphur

ACTION (1) The first effect observed following the injection of growth hormone is nitrogen retention and an increase in body protein Calcium balance remains unchanged

(2) Growth hormone supervises those metabolic activities which are concerned with bringing about the increase in size and weight normally exhibited by young animals It is further respon sible for controlling the proportionate growth of the several organs and parts of the body It is not however the only factor involved in the control of cell multiplication For example, hypophysec tomy does not prevent the compensatory hypertrophy of one kid ney after its fellow has been removed Furthermore the ultimate size attained by a growing animal is determined by other factors such as the capacity of the tissues to respond to growth hormone this response is controlled in part at least by genes

The growth of young animals involves the manufacture of pro teins for the building of new cells This is reflected chemically by the retention of nitrogen water and electrolytes and by a relatively low body content of fat These changes are associated with a food intake which is large in proportion to body weight In the fully grown animal this state of affairs is replaced by a dynamic equili brium in which the intake and the output of nitrogen and minerals become equal and the food intake falls in proportion to the body weight Such increases in body mass which result in a high con tent of water, protein and minerals and a low content of fat are characteristic of true growth as opposed to simple increase in weight

The exact mechanism by which growth hormone affects protein synthesis is unknown but studies with isotopically labelled amino acids suggest that the hormone exerts its effect upon extracellular amino acids impeding their catabolism and promoting their incor poration into tissue proteins

(3) Growth hormone stimulates the activity of the zone of periosteal ossification in long bones causes proliferation of the cartilage of the epiphysial disc (page 241) and in general pro motes every aspect of normal osteogenesis at the junction of the epiphysis and diaphysis[2] It also causes an increase in the turn-

over of phosphorus and calcium by the bone (without affecting the calcium metabolism of the body as a whole)

(4) Specific metabolic effects[2] No doubt the many changes in body metabolism which are observed in animals treated with growth hormone are isolated examples of the overall action of the hormone The following changes have been observed in such animals

(a) Decrease of the urinary excretion of phosphorus

(b) Elevation of inorganic phosphorus and alkaline phosphatase concentrations of the plasma

(c) Decrease of the free amino acid content of the plasma

(d) Increase in protein and water content of the whole animal and a fall in fat content (indicating true growth as opposed to simple increase in bulk[2])

(e) Increase in liver ribonucleic acid and in liver fat during fasting

(f) Hypertrophy of the thymus

It should be noted that growth hormone does not bring about closure of the epiphyses This results from the complex integration of a number of factors chief among which are the sex hormones As long as the epiphyses remain open growth hormone is capable of promoting increase in the length of long bones and hence an increase in longitudinal growth of the body

(5)[3] Young has shown that injections of growth hormone produce diabetes in carnivorous animals after they have reached maturity In other words the hormone produces growth in young animals and diabetes in adult animals The diabetogenic effect of growth hormone is complex and difficult to analyse however it appears to depend upon the following actions of the hormone —

(a) Growth hormone opposes the action of hexokinase which stimulates the phosphorylation of glucose this action of hexokinase is promoted by insulin

(b) *At first growth hormone stimulates the beta cells of the* pancreas to produce more insulin but not sufficient to cope with the increased demand consequent upon the first action

(c) Later the hormone causes atrophy of the beta cells and permanent diabetes follows

(d) The alpha cells of the pancreas are unaffected and continue to produce a hyperglycaemic factor (glucagon) after the beta cells have been destroyed

Recent experiments using highly purified preparations of growth hormone have shown that this diabetogenic action is an inherent property of the hormone and that any chemical changes in the structure of growth hormone which affect one of these two actions also affect the other to the same degree[14]

(6) Growth hormone is an important factor in the maintenance of established lactation (page 136)

The thyroid hormone is thought to synergise the activity of growth hormone whereas the sex hormones appear to antagonise it The fate of growth hormone in the body and the way in which it acts remain quite obscure Although growth hormone is active in certain animals no *preparation used in man* so *far has* produced increase in height Recent studies with a preparation of growth hormone obtained from the human pituitary at autopsy give promise of showing the same effects as the preparations *used* with other species It is, however too soon to comment upon these studies which are at present in progress

Among the theories which attempt to explain the mechanism by which *growth hormone* produces its *many actions* two have received most support Levin believes that the primary action of the hormone is to mobilize fat an action which provides the energy *needed for the retention of nitrogen* Young on the other hand holds that the principal action of growth hormone is the inhibition of the catabolism of protein and carbohydrate the increase in fat metabolism is secondary to this action Both theories hold that growth and the production of fat by the tissues are *fundamentally* opposed to each other

CONTROL OF GROWTH HORMONE SECRETION So far it has not been possible to bring the control of growth hormone secretion into line with that of other pituitary hormones because *tissue growth* produces *no* known hormone which could act in turn upon the pituitary gland and so establish a self regulating mechanism There is however, some evidence to support the idea *that the hypothalamus controls* the secretion of growth hormone thi evidence is based upon studies of the diabetogenic action of the hormone[7]

ASSAY A number of methods are in current use for the assay of growth hormone Of these the most sensitive *involves measurement* of the widening of the proximal tibial epiphysial cartilage of the young hypophysectomised rat treated with *extracts* containing an unknown amount of the hormone

2 THYROID STIMULATING HORMONE

CHEMISTRY TSH has not been isolated in pure form It is a glycoprotein of molecular weight about 10 000 and contains 12 per cent nitrogen

ACTION The principal action of thyroid stimulating hormone as its name implies is to promote thyroid gland secretion This effect is complex and involves the stimulation of all the activities of the thyroid gland which lead to the release of the hormone into the blood In addition thyroid stimulating hormone exerts certain effects outside the thyroid gland

(1) *Thyroid stimulation* These effects occur only in the presence of a normal thyroid gland

(a) Increase in intracellular colloid[5] This change which involves the appearance of droplets within the thyroid cells is seen within 15 minutes of injection of TSH It results from the stimulating effect of TSH upon proteolytic enzyme systems present in the colloid these enzymes split the thyroglobulin molecule into smaller fractions including the thyroid hormones This is perhaps the fundamental action of TSH

(b) Increase in the rate of discharge of the thyroid hormone into the circulation

(c) Increased uptake of iodine by the gland[6]

(d) Increase in the rate of thyroid hormone synthesis This effect is seen 48 hours after an injection of TSH In hypophysectomised animals the rate of conversion of diiodotyrosine to thyroxine is slower than in control animals These responses to TSH are associated with anatomical and histological changes in the thyroid gland —

(i) Increase in the height of the thyroid cells and when this reaches a maximum increase in their number This change begins about 18 hours after an injection of TSH

(ii) Increase in the weight of the gland

As a result of these effects injection of TSH may produce the signs of hyperthyroidism in mammals and accelerates the process of metamorphosis in amphibia

(2) *Systemic effects* These changes which may be produced in the absence of thyroid tissue have been reported in experimental animals following injections of TSH their significance in human physiology and disease remains uncertain

(a) *Orbit* Following injections of TSH in experimental animals there occurs an increase in the fat and water content of orbital structures The extraocular muscles become swollen and

hypertrophic showing fragmentation and lymphocytic infiltration These changes eventually produce protrusion of the eyeballs (exophthalmos)

(b) *Miscellaneous effects*

(i) Water retention

(ii) Increase in plasma fat and blood acetone

(iii) Increase in the number of circulating polymorphonuclear cells—these cells showing fat droplets in their cytoplasm

(iv) Skeletal muscles lose striations and show fat droplets within the muscle cells

The significance of the systemic actions of TSH in normal individuals is not understood Some workers have sought to resolve this riddle by postulating the existence of two hormones one of which exerts the thyroid stimulating effects the other having a systemic action affecting especially the orbital contents It may be said by way of summary that TSH stimulates the thyroid gland to release and to produce greater quantities of its hormones the actions of the hormones which the thyroid gland produces in response to this stimulation have been described in Chapter III Those who hold that there are two hormones prefer to call that which causes exophthalmos EPS (exophthalmos producing substance)

CONTROL OF TSH SECRETION' The self regulating reciprocity which exists between the thyroid and the adenohypophysis has already been described (page 67) A balance is achieved between the circulating levels of thyroxine and TSH whereby an increase in the blood level of thyroid hormone produces a fall in the rate of release of thyrotrophic hormone from the adenohypophysis This state of equilibrium may be affected by a number of factors (Fig 13)

(i) *Stress* Exposure to stress causes a fall in the rate of release of thyrotrophin from the pituitary gland However in the event of prolonged or repeated application of stressor stimuli to experimental animals there follow the phenomenon of escape or adaptation that is to say the concentration of TSH in the blood eventually returns to its previous level in the face of continued exposure to stress It has been shown that both adrenaline and corticosteroids cause a depression of TSH secretion by direct action upon the pituitary gland hence it seems possible that the initial depression of TSH secretion during exposure to

stress may result from the action of adrenaline and corticosteroids which are secreted in response to stressor stimuli

(ii) *Reciprocal relationship between the secretion of TSH and ACTH* In general it can be stated that exposure to stressor stimuli excites the adrenal cortex and inhibits the thyroid gland[4] This response has been shown to reflect a reciprocal relationship between the rates of secretion of ACTH and TSH the former being stimulated the latter depressed[5] Exposure to cold is exceptional among stressor stimuli in that it excites the secretion of both ACTH and TSH whereas the administration of cortisone depresses that of both

(iii) *Hypothalamus and TSH secretion* Until recently it was believed that the effect of thyroid hormone upon the release of TSH was mediated through the adenohypophysis There is good evidence however to show that the blood level of thyroid hormone may effect the release of TSH by way of the hypothalamus rather than by direct action upon the cells of the pituitary The hypothalamus responds to changes in the blood level of thyroid hormone by affecting the function of the adenohypophysis

It has also become clear that stressor stimuli affect the release of TSH by way of the hypothalamus Harris[5] has suggested that certain types of stress (which he calls neural stress) including psychological stress operate by way of the hypothalamus other stressor agents (systemic stress) which affect the metabolic processes of the body act not only by way of the hypothalamus but also by exercising a direct effect upon the cells of the adeno hypophysis The way in which the hypothalamus in turn influences the function of the adenohypophysis is discussed in Chapter XIII where it will be shown that this influence depends upon the extra ordinary nature of the blood supply to the pituitary gland

It is difficult to synthesise these several influences upon thyroid function into a single account of the control of this gland in the normal animal It seems probable that under conditions of relative stability the rate of release of TSH from the adenohypophysis is largely controlled by the concentration of thyroid hormone in the blood Upon this stable background the influence of various stressor stimuli is exerted and these operate by way of the hypothalamus

ASSAY The concentration of TSH in blood can be esti mated by the injection of an extract of plasma into chicks and measuring one of a number of changes produced in the thyroid gland Thyroid weight mitotic figures in thyroid cells and the

hypertrophic showing fragmentation and lymphocytic infiltration These changes eventually produce protrusion of the eyeballs (exophthalmos)

(b) *Miscellaneous effects*

(i) Water retention

(ii) Increase in plasma fat and blood acetone

(iii) Increase in the number of circulating polymorpho nuclear cells—these cells showing fat droplets in their cytoplasm

(iv) Skeletal muscles lose striations and show fat droplets within the muscle cells

The significance of the systemic actions of TSH in normal indi viduals is not understood Some workers have sought to resolve this riddle by postulating the existence of two hormones one of which exerts the thyroid stimulating effects the other having a systemic action affecting especially the orbital contents It may be said by way of summary that TSH stimulates the thyroid gland to release and to produce greater quantities of its hormones the actions of the hormones which the thyroid gland produces in response to this stimulation have been described in Chapter III Those who hold that there are two hormones prefer to call that which causes exophthalmos EPS (exophthalmos producing sub stance)

CONTROL OF TSH SECRETION' The self regulating reciprocity which exists between the thyroid and the adenohy pophysis has already been described (page 67) A balance is achieved between the circulating levels of thyroxine and TSH whereby an increase in the blood level of thyroid hormone pro duces a fall in the rate of release of thyrotrophic hormone from the adenohypophysis This state of equilibrium may be affected by a number of factors (Fig 13)

(i) *Stress* Exposure to stress causes a fall in the rate of release of thyrotrophin from the pituitary gland However, in the event of prolonged or repeated application of stressor stimuli to experimental animals there follow the phenomenon of escape or adaptation that is to say the concentration of TSH in the blood eventually returns to its previous level in the face of con tinued exposure to stress It has been shown that both adrenaline and corticosteroids cause a depression of TSH secretion by direct action upon the pituitary gland hence it seems possible that the initial depression of TSH secretion during exposure to

mechanisms whereby stress induces an increase in ACTH secretion Three views have been put forward to account for this change in the secretion of ACTH —

(i) ACTH secretion is regulated by the systemic blood level of the adrenocortical hormones (ii) ACTH is regulated by the systemic blood level of adrenaline and (iii) ACTH secretion is controlled by the hypothalamus acting through the hypophysial portal vessels These views are not necessarily mutually exclusive

(i) *Systemic blood level of adrenocortical hormones* Arguing by analogy with other trophic hormones Sayers believes that one factor regulating ACTH secretion is the blood level of adreno

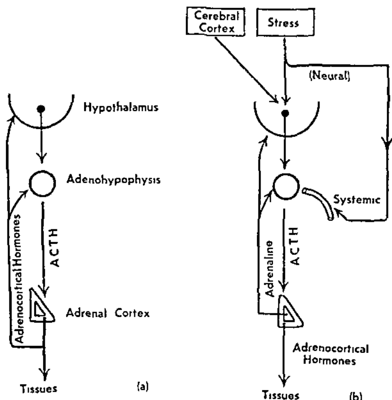


Fig 38 (*a*) Factors regulating the secretion of ACTH under basal conditions

(*b*) Factors responsible for the increased secretion of ACTH under conditions of stress (*G W Harris*)

Two forms of stress (neural and systemic) are shown Neural stress includes most forms of stressor stimuli due to changes in the environment while systemic stress includes conditions which alter the chemistry of the blood

number of intracellular droplets have all been used Recently a number of workers have obtained consistent results using the uptake of radioactive iodine by the chick thyroid before and after injection of extracts containing TSH Other workers use rodents for TSH estimations and their results using methods previously applied to chicks suggest that the rodent gives more consistent results

3 ADRENOCORTICOTROPHIC HORMONE

CHEMISTRY The composition of ACTH has proved elusive It was thought to be a protein but most investigators believe that the active component of protein extracts is probably a polypeptide There may be two chemically distinct ACTH s but at the present time most workers believe that only one hormone exists

ACTION ACTH stimulates the activity of the adrenal cortex and continued administration may lead to hypertrophy and finally to the development of cortical adenomata this hypertrophy chiefly affects the zona fasciculata It will be recalled that adrenocortical steroids fall into 3 main groups androgens glucocorticoids and mineralocorticoids (page 35) ACTH does not appear to stimulate mineralocorticoid activity in man and although it increases the production of adrenal androgens, little is known of the conditions under which it does so However there can be no doubt that ACTH increases the production of glucocorticoids and it may be that it does this by stimulating the conversion of cholesterol to pregnenolone (page 43) This reaction appears to be the fundamental pacemaker of adrenocortical activity at least as far as the production of corticosteroids is concerned This is the only known direct action of ACTH The effects which result from glucocorticoid and androgen secretion have been described elsewhere (see Chapter II)

CONTROL OF ACTH SECRETION[9] The part which variations in the secretion of ACTH play in regulating adrenocortical activity have already been discussed (page 45) Here it is proposed to deal with the mechanisms involved in these variations of ACTH output by the adenohypophysis

Efforts to separate the quiescent state of the adrenal cortex from its activity under conditions of stress have developed into sterile academic considerations chiefly centred about the definition of such a state of quiescence which in the last analysis is largely a theoretical concept More rewarding have been studies of the

ASSAY[11] Two principal methods for the estimation of ACTH are in use and a number of workers believe that each method measures a different hormone i e that there are two distinct ACTH hormones[12] The first method measures the effect of ACTH upon the weight of the adrenal cortex (adrenal weight factor) while the second measures the effect of ACTH upon the content of ascorbic acid in the cortex (ascorbic acid factor) These methods are performed upon hypophysectomised animals and are laborious A third method which measures the effect of ACTH upon the nestling rat thymus has been used more frequently in recent years[1]

GONADOTROPHIC HORMONES

4 FOLLICLE STIMULATING HORMONE (FSH)

CHEMISTRY FSH is a glycoprotein with an isoelectric point at about pH 4 8

ACTION In the female so far as is known the only action of FSH is upon the ovary It influences the accessory sexual apparatus only by way of the gonads The action of FSH upon the ovary is chiefly morphological It converts the primordial follicle of one menstrual cycle into a Graafian follicle during the luteal phase of the previous cycle By the time of onset of menstruation an antrum has formed in the follicle which is to feature in the succeeding menstrual cycle (Chapter V) In the male FSH stimulates and maintains spermatogenesis

METABOLISM FSH is excreted in the urine and it is probable that urinary levels reflect blood levels

ASSAY Urinary levels of FSH are estimated by injecting extracts of urine into immature female mice or rats The criteria of response commonly used are (a) microscopic changes in the ovary or vagina (b) increase in weight of the uterus or ovary

5 LUTEINIZING HORMONE (LH)

CHEMISTRY LH is a glycoprotein of molecular weight about 90 000 and an isoelectric point at about pH 7 45

ACTION In the female luteinizing hormone is necessary for the preovulatory enlargement of the ovarian follicle for ovulation and for the formation of the corpus luteum However its chief function is to promote the secretion of oestrogens and of progesterone In the male luteinizing hormone is called interstitial cell

cortical steroids It has been suggested that the Sayers hypothesis may account for a steady base line output of ACTH against the background of which other factors adjust the pituitary activity to the needs of the moment (Fig 38)

(ii) *Systemic blood level of adrenaline* Long and his colleagues have produced evidence to show that another factor responsible for increased ACTH secretion under conditions of stress is the rise in blood level of adrenaline which is characteristic of such states It is probable that adrenaline exerts this effect by acting directly upon the cells of the anterior pituitary gland and upon the hypothalamus (Fig 38)

(iii) *Hypothalamic control* A great deal of experimental work supports the view that the hypothalamus is important in ACTH secretion[9] It may be stated that stressor stimuli affect the hypothalamus which in turn alters the activity of the anterior pituitary The way in which the hypothalamus exerts this effect forms the subject of a later chapter (Chapter XIII) but stated briefly it is believed to be by way of the extraordinary blood supply to the pituitary gland

In addition to the mechanisms which account for ACTH secretion in response to stressor stimuli recent experiments have suggested that the cerebral cortex is capable of influencing ACTH secretion[9]

Summary of the control of ACTH secretion (Fig 38)

1) Under optimum conditions of stable environment the main stimulus to ACTH secretion appears to be some basal hypothalamic activity operating via the hypophysial portal vessels This base line secretion is also in part the result of the level of adrenocortical hormones in the systemic blood

2) Under conditions of stress it seems likely that both of these mechanisms play a part in controlling the rate of secretion of ACTH Some forms of stress (neural stress) operate by way of nerve reflex paths which act through the hypothalamus and the hypophysial portal vessels Other forms of stress (systemic stress), act both by way of the hypothalamus and by producing changes in the blood level of corticosteroids which directly affect the anterior pituitary gland It seems probable that the secretion of adrenaline may play a subsidiary role in exciting ACTH secretion The level of adrenocortical steroids in the systemic blood is less important in the secretion of ACTH during conditions of stress than changes in the hypothalamic control of the pituitary gland

ASSAY[11] Two principal methods for the estimation of ACTH are in use and a number of workers believe that each method measures a different hormone i e that there are two distinct ACTH hormones[12] The first method measures the effect of ACTH upon the weight of the adrenal cortex (adrenal weight factor) while the second measures the effect of ACTH upon the content of ascorbic acid in the cortex (ascorbic acid factor) These methods are performed upon hypophysectomised animals and are laborious A third method which measures the effect of ACTH upon the nestling rat thymus has been used more frequently in recent years[1]

GONADOTROPHIC HORMONES

4 FOLLICLE STIMULATING HORMONE (FSH)

CHEMISTRY FSH is a glycoprotein with an isoelectric point at about pH 4 8

ACTION In the female so far as is known the only action of FSH is upon the ovary It influences the accessory sexual apparatus only by way of the gonads The action of FSH upon the ovary is chiefly morphological It converts the primordial follicle of one menstrual cycle into a Graafian follicle during the luteal phase of the previous cycle By the time of onset of menstruation an antrum has formed in the follicle which is to feature in the succeeding menstrual cycle (Chapter V) In the male FSH stimulates and maintains spermatogenesis

METABOLISM FSH is excreted in the urine and it is probable that urinary levels reflect blood levels

ASSAY Urinary levels of FSH are estimated by injecting extracts of urine into immature female mice or rats The criteria of response commonly used are (a) microscopic changes in the ovary or vagina (b) increase in weight of the uterus or ovary

5 LUTEINIZING HORMONE (LH)

CHEMISTRY LH is a glycoprotein of molecular weight about 90 000 and an isoelectric point at about pH 7 45

ACTION In the female luteinizing hormone is necessary for the preovulatory enlargement of the ovarian follicle for ovulation and for the formation of the corpus luteum However its chief function is to promote the secretion of oestrogens and of progesterone In the male luteinizing hormone is called interstitial-cell

cortical steroids It has been suggested that the Sayers hypothesis may account for a steady base line output of ACTH against the background of which, other factors adjust the pituitary activity to the needs of the moment (Fig 38)

(ii) *Systemic blood level of adrenaline* Long and his colleagues have produced evidence to show that another factor responsible for increased ACTH secretion under conditions of stress is the rise in blood level of adrenaline which is characteristic of such states It is probable that adrenaline exerts this effect by acting directly upon the cells of the anterior pituitary gland and upon the hypothalamus (Fig 38)

(iii) *Hypothalamic control* A great deal of experimental work supports the view that the hypothalamus is important in ACTH secretion[8] It may be stated that stressor stimuli affect the hypothalamus which in turn alters the activity of the anterior pituitary The way in which the hypothalamus exerts this effect forms the subject of a later chapter (Chapter XIII) but stated briefly it is believed to be by way of the extraordinary blood supply to the pituitary gland

In addition to the mechanisms which account for ACTH secretion in response to stressor stimuli recent experiments have suggested that the cerebral cortex is capable of influencing ACTH secretion[9]

Summary of the control of ACTH secretion (Fig 38)

1) Under optimum conditions of stable environment the main stimulus to ACTH secretion appears to be some basal hypothalamic activity operating via the hypophysial portal vessels This base line secretion is also in part the result of the level of adreno cortical hormones in the systemic blood

2) Under conditions of stress it seems likely that both of these mechanisms play a part in controlling the rate of secretion of ACTH *Some forms of stress (neural stress) operate by way of nerve reflex paths which act through the hypothalamus and the hypophysial portal vessels Other forms of stress (systemic stress), act both by way of the hypothalamus and by producing changes in the blood level of corticosteroids which directly affect the anterior pituitary gland It seems probable that the secretion of adrenaline may play a subsidiary role in exciting ACTH secretion The level of adrenocortical steroids in the systemic blood is less important in the secretion of ACTH during conditions of stress than changes in the hypothalamic control of the pituitary gland*

the central nervous system which controls the adenohypophysis by way of the hypothalamus (Fig 39)

A number of environmental factors are known to influence gonadotrophic activity and present evidence suggests that they operate through the central nervous system In this way the adeno hypophysis acts as a buffer between the central nervous system and the gonads[10] (Fig 39) Among the environmental factors which are capable of influencing gonadotrophic activity in animals the following have been studied closely —food temperature light the presence of eggs of young and of a companion of the same sex or a sterile mate In addition to these environmental factors stimulation of the internal and external genital organs in some species can bring about changes in the rate of gonado trophic secretion[10]

The importance of the hypothalamus in the control of gonado trophic secretion is shown by the fact that electrical stimulation of the tuber cinereum brings about ovulation in experimental animals while electrical stimulation of the pituitary gland itself *fails to do so Pharmacological stimulation of the hypothalamus* (by means of such agents as copper salts picrotoxin and metrazol) will cause ovulation only if the pituitary stalk is intact[10] These and other experiments suggest that gonadotrophic secretion depends upon hypothalamic stimulation of the adenohypophysis As with other trophic hormones the basal level of secretion depends upon the blood levels of hormones secreted by the target glands (in this case the gonads) A fall in the level of a particular target hormone stimulates the production of the appropriate trophic hormone as explained in Chapters IV and V Upon this basal level of activity various environmental and other factors operate These mechan isms appear to act chiefly by way of the hypothalamus rather than directly upon the pituitary gland

ASSAY Urinary assays of LH or ICSH have been attempted by a number of methods but so far the estimation of this hormone remains unsatisfactory

6 PROLACTIN (LACTOGENIC HORMONE OR LUTEOTROPHIC HORMONE)

CHEMISTRY Prolactin is a protein which is free of carbo hydrate It has an isoelectric point of pH 5 65

ACTION The significance of prolactin in the male is not understood In the female it has a twofold action which is respon sible for its two names In the first place it stimulates the breast

stimulating hormone (ICSH)* and is responsible for the stimulation of the interstitial cells of the testis which results in the production of testicular androgens The actions of the hormones resulting from gonadotrophic stimulation of the testis and the ovary have been discussed in Chapters IV and V respectively

METABOLISM LH is excreted in the urine together with FSH The relative proportions of the two hormones in urine varies throughout the menstrual cycle but the details of this variation are not known because assay of LH is a difficult procedure

CONTROL OF GONADOTROPHIC SECRETION The self-regulating mechanism whereby gonadotrophins stimulate the production of hormones from the gonads which in turn depress the secretion of the gonadotrophins themselves has been outlined in Chapters IV and V It is now generally believed that the hormones secreted by the gonads influence gonadotrophic secretion by means of their effect upon the central nervous system, rather than by a direct action upon the adenohypophysis It may be that the hormones of the gonads do have some direct effect upon the pituitary gland itself but in general this is overshadowed by the action upon

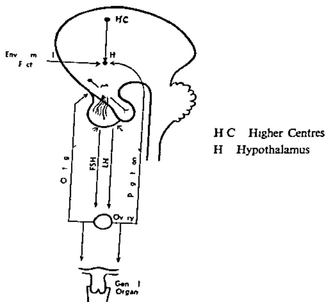


Fig 39 The control of the secretion of gonadotrophins (*After G W Harris*)

* The term interstitial cell stimulating hormone is sometimes applied to the hormone in either sex and some authors have abandoned the term luteinizing hormone

the central nervous system which controls the adenohypophysis by way of the hypothalamus (Fig 39)

A number of environmental factors are known to influence gonadotrophic activity and present evidence suggests that they operate through the central nervous system In this way the adenohypophysis acts as a buffer between the central nervous system and the gonads[10] (Fig 39) Among the environmental factors which are capable of influencing gonadotrophic activity in animals the following have been studied closely —food temperature light the presence of eggs of young and of a companion of the same sex or a sterile mate In addition to these environmental factors stimulation of the internal and external genital organs in some species can bring about changes in the rate of gonadotrophic secretion[10]

The importance of the hypothalamus in the control of gonadotrophic secretion is shown by the fact that electrical stimulation of the tuber cinereum brings about ovulation in experimental animals while electrical stimulation of the pituitary gland itself fails to do so Pharmacological stimulation of the hypothalamus (by means of such agents as copper salts picrotoxin and metrazol) will cause ovulation only if the pituitary stalk is intact[1] These and other experiments suggest that gonadotrophic secretion depends upon hypothalamic stimulation of the adenohypophysis As with other trophic hormones the basal level of secretion depends upon the blood levels of hormones secreted by the target glands (in this case the gonads) A fall in the level of a particular target hormone stimulates the production of the appropriate trophic hormone as explained in Chapters IV and V Upon this basal level of activity various environmental and other factors operate These mechanisms appear to act chiefly by way of the hypothalamus rather than directly upon the pituitary gland

ASSAY Urinary assays of LH or ICSH have been attempted by a number of methods but so far the estimation of this hormone remains unsatisfactory

6 PROLACTIN (LACTOGENIC HORMONE OR LUTEOTROPHIC HORMONE)

CHEMISTRY Prolactin is a protein which is free of carbohydrate It has an isoelectric point of pH 5 65

ACTION The significance of prolactin in the male is not understood In the female it has a twofold action which is responsible for its two names In the first place it stimulates the breast

an action which is especially important during pregnancy In the second place prolactin stimulates the corpus luteum It is tempting to suppose that these two diverse actions are due to two different hormones but so far as is known prolactin and luteotrophin are one and the same In addition to these two actions certain other effects are observed following the administration of preparations of prolactin to experimental animals Whether these additional effects can be ascribed to the direct action of the hormone itself remains uncertain

1) *Breast*

(a) *Breast development* Prolactin is capable of promoting the growth of breast tissue in a breast already prepared by the action of oestrogens and progesterone This action of prolactin is particularly important during the last trimester of pregnancy

(b) *Lactation* Prolactin promotes and maintains lactation This action is probably a direct one upon the breast which has been prepared by the action of oestrogens and progesterone The process of lactation requires the presence of normal adrenocortical and thyroid function although the exact role of these two functions in the process of lactation is not yet clear This aspect of the action of prolactin is discussed in Chapter VI

2) *Luteotrophic action* Prolactin maintains the corpus luteum once it has developed and in this way stimulates the secretion of progesterone In the absence of prolactin the corpus luteum develops but soon undergoes atrophy When this action of prolactin was thought to be due to a separate hormone the name luteotrophic hormone was coined it is now believed that this effect is due to prolactin so that the term luteotrophic hormone is better avoided

3) *Maternal instinct* In some animals it is possible to show that prolactin stimulates the maternal instinct Virgin animals treated with prolactin begin to care for younger animals and to display maternal behaviour

4) *Miscellaneous* Prolactin produces an increase in body metabolism and can produce diabetes in animals These effects are not important under physiological conditions

CONTROL OF PROLACTIN SECRETION[11] In the study of lactation it is important to bear in mind that whereas prolactin is responsible for the final phase of breast activity which culminates in the secretion of milk this function is quite distinct from that of milk ejection which is controlled by the posterior pituitary

gland (Fig 40) The nervous stimulation of suckling is the important factor in initiating and maintaining the secretion of prolactin and hence the secretion of milk The essential element of suckling is the sensory stimulus to the nipple[11] The central pathways of the suckling stimulus appear to involve ascending spinal fibres

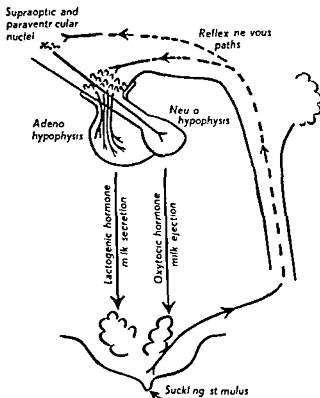


Fig 40 The control of lactation (*G W Harris*)

together with the hypothalamus and pituitary stalk (Fig 40) a motor nerve supply to the mammary gland is not essential In other words we are dealing with a neurohumoral reflex—nervous on its afferent side and humoral on its efferent side The afferent spinal fibres probably stimulate the hypothalamus which in turn acts on the adenohypophysis by way of the hypophysial portal vessels (page 252) The possibility that oxytocin may affect the secretion or release of prolactin is discussed on page 136

ASSAY It is possible to estimate urinary levels of prolactin from the effect produced by the injection of extracts upon the weight of the crop glands of pigeons of two or three months of age

7 MELANOCYTE STIMULATING HORMONE (INTERMEDIN OR MSH)

MSH is probably secreted by the pars intermedia, which is poorly developed in man

CHEMISTRY Little is known of the chemistry of MSH It is a protein and is quite distinct from ACTH The separation of these two hormones proved to be very difficult with the result that the properties of MSH were originally attributed to ACTH

ACTION MSH causes pigment dispersion in the chromatophores of the skin of cold blooded vertebrates and hence produces darkening of the skin prolonged administration of the hormone causes a true increase in the melanin content of skin This action of MSH brings about rapid changes in the skin colour in response to various stimuli

Although MSH is certainly present in the mammalian pituitary its function in such animals is not clear It cannot bring about rapid dispersion of pigment within the melanocytes of the mammalian skin Nevertheless the hormone is thought to exert some influence upon the formation of melanin in mammals and it has been suggested that very slow dispersion of melanin granules within the melanocytes of certain mammalian species may result from the action of MSH Alternatively it has been stated that the hormone can affect the colour of mammalian skin by activating the enzyme tyrosinase Tyrosinase promotes the oxidation of tyrosine to 3 4-dihydroxyphenylalanine (DOPA) (page 182) and the latter can be converted to melanin in the skin It is hoped that recent advances in the assay of MSH will clarify its function in man

CONTROL Little is known about the control of MSH secretion but it appears likely that cortisone or hydrocortisone depresses the secretion of the hormone by the pituitary (Fig 41)

ASSAY It is possible to estimate the amount of MSH in blood by a number of methods which involve measurement of melanophore expansion or changes of colour produced in the skin of the frog following injection of extracts of blood

ENDOCRINE FACTORS IN THE CONTROL OF SKIN PIGMENTATION The details of factors involved in human skin

pigmentation are very incompletely understood It seems clear that MSH is partly responsible for stimulating melanocyte *activity and equally clear that the release of this hormone from* the pituitary gland is depressed by glucocorticoid secretion from the adrenal cortex[15] In animals it has been shown that the effect of MSH upon melanocytes can be diminished by adrenaline and nor adrenaline[15] but this effect is less clearly established in the case of man (Fig 41) This *action of the catecholamines can be*

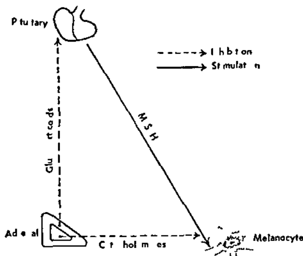


Fig 41 Diagram showing the action of hormones upon the melanocytes of the skin MSH stimulates the melanocyte adrenaline and nor adrenaline interfere with this action of MSH while glucocorticoids depress the release *of MSH from the pituitary gland*

blocked by sympatholytic agents such as dibenamine Oestrogens appear to stimulate melanocytes in man while progesterone has a similar effect in certain animals Thyroid hormone can cause the skin of certain species to assume a lighter colour but as in the case of progesterone this effect is not so readily demonstrated in man[15]

Histology in Relation to Function

Until recently it was generally agreed that the chromophobe cells of the adenohypophysis do not secrete active hormones It is therefore an intriguing problem to allot the secretion of six hor mones to two cell types (alpha and beta) Some workers have endeavoured to overcome this difficulty by postulating the separate

existence of only two hormones one secreted by each type of cell It is suggested that these two hormones consist of large complex molecules which the appropriate end organ uses for its own needs For example one molecule might share the actions of ACTH and TSH The thyroid would make use of one part of the molecule and the adrenal cortex of another The six (excluding MSH) relatively pure hormone preparations in existence would then constitute artificial products of chemical dissection

More generally it is held that FSH LH and TSH are derived from the beta cells while growth hormone and prolactin come from the alpha cells The exact origin of ACTH is still disputed and the previously accepted idea that it was secreted by the beta cells has been challenged

As the result of recent histochemical studies in dogs it has been realised that more than two secreting cell types exist in the adenohypophysis Purves and Griesbach[13] have not only described six types of cells in the gland but as the result of experiments in which animals were treated with oestrogens thyroxine and other hormones these workers have produced evidence to show that the cells of the adenohypophysis secrete hormones in the following way —

(I) *Orange acidophils* → *Growth hormone*

(II) *Red acidophils* → *Prolactin*

(III) *Blue basophils* → *Thyroid stimulating hormone*

(IV) *Pale basophils* → *FSH and LH (or ICSH)*

The cells of the pars intermedia presumably secrete MSH while the site of ACTH secretion remains obscure Evidence at present available suggests that neither acidophil nor basophil cells secrete ACTH and it is therefore presumed that the chromophobe cells are responsible for the production of this hormone *Although* these observations have not been confirmed in man there is some indirect evidence to suggest that essentially the same state of affairs prevails in the human adenohypophysis

DISEASES OF THE ADENOHYPOPHYSIS

Three diseases of the gland can be mentioned in passing because they illustrate the functions of the adenohypophysis

ACROMEGALY AND GIANTISM The alpha cells of the adenohypophysis may give rise to the formation of adenomata and thereby produce clinical evidence of overactivity *Before puberty*

affected individuals grow into well proportioned giants After maturity increase in the size of the hands and feet together with massive development of the bones of the face occur This condition is called acromegaly and demonstrates the effect of growth hormone in excessive amounts

CUSHING S SYNDROME Some cases of this condition appear to result from adenomata of the beta cells of the pituitary gland The clinical features of this syndrome represent the picture of excessive production of glucocorticoids and androgens (especially the former) This is the pathological counterpart of prolonged cortisone therapy in chronic diseases such as rheumatoid arthritis The condition appears to represent an excessive secretion of ACTH and was responsible for the original suggestion that this hormone was elaborated by the beta cells

HYPOPITUITARISM

(i) *Dwarfism* Some individuals fail to reach the lower limits of the normal range of height for individuals of their race and sex One cause of this failure is an isolated deficiency of growth hormone There may also be a defect in the secretion of gonadotrophins in this case the epiphyses remain open as the result of failure on the part of the gonads to secrete their hormones which are the chief factors responsible for closing the epiphyses

(ii) *Hypogonadism in males* Isolated failure of gonadotrophic secretion is sometimes seen in men Under these conditions the epiphyses remain open through failure of secretion of testicular androgens However the secretion of growth hormone remains normal and so continues to stimulate the growth of long bones with the result that affected individuals become tall and develop very long limbs

(iii) *Panhypopituitarism* After parturition it sometimes happens that failure of the adenohypophysis involving all its functions sets in This failure is not uncommon when excessive blood loss has occurred during labour and appears to result from damage to the hypophysial blood supply which at that time appears to be especially vulnerable to such damage Under these circumstances failure of all trophic hormones becomes evident Failure of lactation failure of menstrual cycles to re-establish themselves signs of hypothyroidism and sometimes evidence of adrenocortical failure may all appear In the absence of therapeutic preparations of all the trophic hormones cortisone thyroid extract and oestrogens are used to substitute for the natural products of the target

existence of only two hormones *one secreted by each type of cell* It is suggested that these two hormones consist of large complex molecules which the appropriate end organ uses for its own needs For example one molecule might share the actions of ACTH and TSH The thyroid would make use of one part of the molecule and the adrenal cortex of another The six (excluding MSH) relatively pure hormone preparations in existence, would then constitute artificial products of chemical dissection

More generally it is held that FSH LH and TSH are derived from the beta cells while growth hormone and prolactin come from the alpha cells The exact origin of ACTH is still disputed and the previously accepted idea that it was secreted by the beta cells has been challenged

As the result of recent histochemical studies in dogs it has been realised that more than two secreting cell types exist in the adenohypophysis Purves and Griesbach[13] have not only described six types of cells in the gland but as the result of experiments in *which animals were treated with oestrogens thyroxine and other* hormones these workers have produced evidence to show that the cells of the adenohypophysis secrete hormones in the following way —

(I)	*Orange acidophils*	→	*Growth hormone*
(II)	*Red acidophils*	→	*Prolactin*
(III)	*Blue basophils*	→	*Thyroid stimulating hormone*
(IV)	*Pale basophils*	→	*FSH and LH (or ICSH)*

The cells of the pars intermedia presumably secrete MSH while the site of ACTH secretion remains obscure Evidence at present available suggests that neither acidophil nor basophil cells secrete ACTH and it is therefore presumed that the chromophobe cells are responsible for the production of this hormone Although these observations have not been confirmed in man there is some indirect evidence to suggest that essentially the same state of affairs prevails in the *human adenohypophysis*

DISEASES OF THE ADENOHYPOPHYSIS

Three diseases of the gland can be mentioned in passing because they illustrate *the functions of the adenohypophysis*

ACROMEGALY AND GIANTISM The alpha cells of the adenohypophysis may give rise to the formation of adenomata and thereby produce clinical evidence of overactivity Before puberty

CHAPTER VIII

THE NEUROHYPOPHYSIS

The neurohypophysis develops from the floor of the third ventricle of the brain it is not strictly speaking an endocrine gland

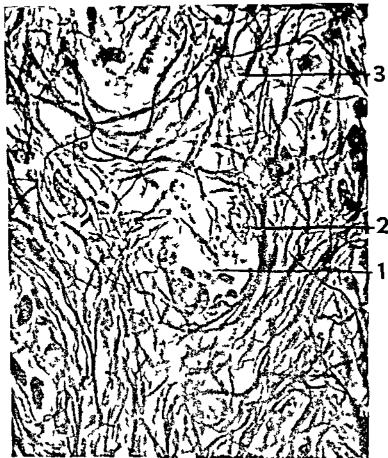


Fig 42 (a) Section of the human neurohypophysis This microphotograph shows the three components of the neurohypophysis 1 Capillary blood vessel 2 Pituicyte 3 Nerve fibres

glands This treatment brings about a remarkable improvement in the condition of such patients

REFERENCES

As in other chapters of this book only certain outstanding references are given A careful survey of the extensive literature dealing with the pituitary gland has been undertaken in "Diseases of the Endocrine Glands by L J Soffer Lea & Febiger Philadelphia Certain aspects of pituitary function especially the control of its secretions is dealt with in great detail with full references to the literature in "Neural Control of the Pituitary Gland by G W Harris Edward Arnold London Methods of assay of pituitary hormones are described in detail in "Hormone Assay" by C W Emmens Academic Press New York 1950

1 Rioch D M Wislocki G B & O'Leary J L Res Publ Ass Nerv Ment Dis XX 3 1940
2 Li C H The Harvey Lectures 46 181 1957 Charles C Thomas Springfield Ill
3 Young F G Biochem J 39 515 1945
4 Young F G Sydney Ringer Lecture 1951
5 De Roberts E N Y As Sci Jan 1947
6 Closs K et al J Biol Chem 96 585 1952
7 Harris G W Neural Control of the Pituitary Gland p 158 Edward Arnold London 1956
8 Harris G W Idem p 132
9 Harris G W Idem p 103
10 Harris G W Idem p 60
11 Harris G W Idem p 153
12 Stack Dunne M & Young F G J Endocrinol 7 66 1951
13 Purves H D and Griesbach W E J Endocrinol 14 361 1957
14 Reid E J Endocrinol 8 50 1952
15 Lerner A B J Clin Endocrinol 14 1463 1954

feature of the histology of the neurohypophysis should be mentioned namely the presence of swellings or thickenings along the course of the nerve fibres These swellings give certain of the neurohypophysial fibres the appearance of a string of beads while similar droplets or granules are found free within the neurohypophysis not immediately related to nerve fibres Some of the swellings along the fibres are the so-called Herring bodies once thought to lie free within the neurohypophysis but recently shown by the electron microscope to occur along the course of the nerve fibres (Fig 43) It will be seen from this brief account of neurohypophysial histology that the neural lobe contains no glandular elements resembling the typical secretory cells of other endocrine structures

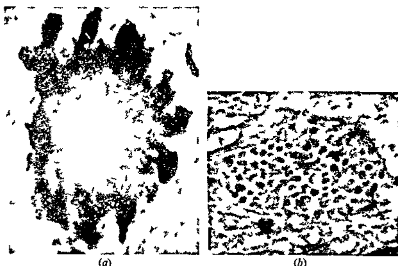


Fig 43 (*a*) Shows a Herring body with protuberances The darkly staining material consists of neurosecretory droplets associated with a nerve fibre (x 1 400)

(*b*) Shows an electron microphotograph of a single nerve fibre in the infundibular process of the cat containing numerous neurosecretory granules (x 23 000) (*W Bargmann*)

Function

It is now generally believed that the function of the neurohypophysis is to store and to release as required two hormones secreted by the nerve cells of the supraoptic and paraventricular nuclei of the hypothalamus The two hormones are called vasopressin or the antidiuretic hormone (ADH) and oxytocin Both

but rather a storehouse for the internal secretions of the hypothalamus. However it is possible that the hormones concerned undergo chemical changes in the neurohypophysis and it is certain that this structure is essential to the normal release of the stored hormones.

Histology

The three important histological components of the neurohypophysis are fine nerve fibres which together form a plexus, fusiform and irregularly shaped cells called pituicytes and capillaries surrounded by argyrophil connective tissue. The pituicytes are glial cells and do not represent modified nerve cells as was once thought. When living these cells contain refractile granules and droplets (Fig 42).

The nerve fibres of the neurohypophysis form a thick network of unmyelinated fibres and in the vicinity of blood vessels give rise to a particularly fine meshed plexus. These fibres do not form synapses with the pituicytes and the vast majority of them are derived from cells in the supraoptic and paraventricular nuclei (Fig 42); a few originate from ganglion cells in the tuber cinereum.

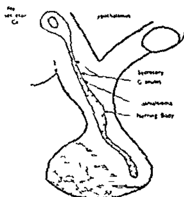


Fig 42 (*b*) Diagrammatic representation of the migration of neurosecretory material within the neuroplasm of a neurosecretory fibre. The origin of the neurosecretory granules in the hypothalamus and their journey along the hypothalamo hypophysial tract is illustrated in a highly diagrammatic form. The congregation of granules forming a Herring body is also shown.

The cells of the supraoptic and paraventricular nuclei show in their cytoplasm droplets of a colloid substance which recent studies have shown to be a product of neurosecretion. One further

depot for this material which it releases according to the requirements of the moment (Fig 42 (b)) The neurosecretory material is almost certainly formed in the supraoptic and paraventricular nuclei from which it is transported down the hypothalamo hypophysial tract to the neurohypophysis The accumulation of this neurosecretory material can be observed above the site of an experimental obstruction of the hypothalamo hypophysial tract[7]

How the neurosecretory material is released into the blood stream or exactly what chemical relationship it bears to the definitive hormones cannot be stated with certainty It seems probable however that the neurosecretion consists of a mixture of the two hormones (ADH and oxytocin) and that it enters the blood stream by crossing the walls of the neurohypophysial capillaries These capillaries have been shown to possess minute pores revealed by the electron microscope pores which resemble those described in the capillaries of the renal glomerulus[7] This observation would seem to suggest that the capillaries of the neurohypophysis could allow products of neurosecretion to pass through their walls into the blood stream

Chemistry

The brilliant researches of du Vigneaud have resulted in the isolation and subsequent synthesis of two similar but distinct polypeptides each of molecular weight of about 1 000 (Fig 2) Present day evidence is strongly in favour of the view that these are the two hormones ADH and oxytocin Previous extracts of neurohypophysial tissue resulted in the isolation of a protein molecule of molecular weight of 30 000 which is now held to be a complex formed by ADH oxytocin and some inert protein The possibility still exists that this protein complex may represent a neurosecretion in some stage of formation more generally it is held to be an artifact of extraction

Each of the pure polypeptides contains eight amino acids of which six are common to both (page 18) Indeed the chemical similarity between the two compounds is quite striking One interesting feature of these hormones is that while pure oxytocin shows no antidiuretic activity ADH possesses a slight degree of oxytocic action Antidiuretic hormone extracted from beef differs from that of hog by one amino acid oxytocin is identical in these two species

names for the antidiuretic hormone are used in current endocrine literature but the term antidiuretic hormone (abbreviated to ADH) will be used here because it describes the most important function of the hormone

Fig 44 Diagram of a sagittal section through the pituitary gland showing the innervation of the neurohypophysis and the distribution of the fibres of the hypothalamo hypophysial tract (H C Thomas by permission of *Journal of Clinical Endocrinology and Metabolism* 195)

Histology in Relation to Function

It is now believed that the granules or droplets seen within the cytoplasm of the nerve cells which comprise the supraoptic and paraventricular nuclei are products of neurosecretion The development of electron microscopy together with the beautiful histological preparations of Bargmann[7] and other workers has enabled investigators in this field to trace these products of neurosecretion from the vicinity of the cell nuclei to the point where the nerve fibres fan out in the neurohypophysis (Fig 44) The Herring bodies and other swellings which give these fibres their beaded appearance represent the neurosecretory material in various parts of its journey to the neurohypophysis similar material is to be found free within the neural lobe

The conception of neurosecretion did not pass unchallenged but it can now be stated that the majority of workers are unanimous in accepting this concept of neurohypophysial function The neural lobe contains far more of this neurosecretory material than any other part of the nervous pathway from which it takes origin and this suggests that the neurohypophysis acts as a storage

mone upon the excretion of sodium and chloride It is likely that changes in sodium and chloride excretion observed after injections of ADH are due to a decrease in mineralocorticoid activity resulting from water retention

OXYTOCIN

Action 1 Oxytocin stimulates the plain muscle of the body of the uterus but does not affect that of the cervix in exerting this action oxytocin is antagonised by progesterone In addition oxytocin has a stimulating effect upon the plain muscle of the intestine gall bladder ureter and urinary bladder but these actions are less marked than in the case of the uterus

The action of oxytocin upon the uterus varies from species to species but in woman it is most marked towards the end of pregnancy This observation suggests that the hormone may play some part in initiating labour How important this action of oxytocin is in the onset of normal labour remains uncertain

2 Oxytocin promotes the ejection of milk by stimulating the plain muscle of the nipple and the myoepithelium of the breast (page 135) In addition to this action oxytocin may play some part in promoting the secretion or release of prolactin by the adenohypophysis (page 135)

Joint Metabolic Effects of ADH and Oxytocin Injections of the impure preparations of the two hormones of the neurohypophysis which have so far been available produce in addition to the major actions characteristic of each certain metabolic effects These changes appear to be of minor importance and are probably of no great significance under physiological conditions

1 *Carbohydrate metabolism* These hormones antagonise the action of insulin and cause —

(i) diminished sugar tolerance

(ii) diminished liver glycogen

(iii) hyperglycaemia

(iv) glycosuria

2 *Tissue metabolism* The hormones cause a fall in the basal metabolic rate this action is more marked with preparations of ADH than with oxytocin

3 *Fat metabolism* An increase in the fat content of the liver may follow repeated injections of posterior pituitary extracts

4 *Muscle metabolism* Extracts of neurohypophysial tissue may cause a temporary anaerobic phase of muscle metabolism

Action

Extracts of neurohypophysial tissue are capable of exerting four main effects namely stimulation of the uterus milk ejection, anti diuretic activity and elevation of blood pressure Of these four actions the first two (stimulation of the uterus and milk-ejection) are held to be properties of the hormone oxytocin while the remaining two actions (antidiuresis and elevation of blood pressure) are due to the hormone ADH or vasopressin

In addition to these major actions the hormones alone and together exert certain metabolic effects when injected into experimental animals The significance of these minor effects in the physiology of normal individuals is at present obscure Furthermore the commercial preparations of the hormones used in earlier experiments were not sufficiently pure to enable observers to attribute each of these miscellaneous effects to one or other of the hormones

ANTIDIURETIC HORMONE

Action 1 ADH restricts the diuresis which follows the ingestion of fluid It acts directly upon the kidney promoting absorption of water from the loop of Henle and the distal convoluted tubule This effect is altered neither by denervation of the kidney nor by changes in renal blood flow[3]

2 *Pressor and vasopressor action* In man injections of ADH usually cause a fall in pulse rate a decline in cardiac output and a decrease in oxygen consumption this fall in cardiac output results from constriction of the coronary vessels Usually there is no change in blood pressure In animals a rise in blood pressure due to the constriction of arterioles and capillaries is seen in some species but the experimental conditions (especially the anaesthetic used) modify the response elicited This action when it occurs is due to a direct effect on the musculature of the blood vessels and is not antagonised by nicotine or by cutting the spinal cord The coronary vasoconstriction however may be obviated by the concomitant administration of adrenaline Recent experiments have shown that ADH brings about the closure of arterio venous connections and it is probable that the pressor activity of the hormone largely depends upon this action[9]

3 *Miscellaneous actions* ADH stimulates intestinal peristalsis and causes hyperglycaemia It is also said to cause an increase in the rate of excretion of chloride in the urine but a good deal of controversy still exists about the action of the hor-

The activity of the neurohypophysis is regulated by the hypothalamus and Harris has pointed out that this relationship is as constant and as intimate as that which exists between a voluntary muscle and the lower motor neurones which supply it For if the hypothalamo hypophysial nerve tracts are severed the neurohypophysis ceases to function and undergoes atrophy[6] Experimental obstruction to the passage of neurosecretory material along these tracts by means of some form of pressure causes an accumulation of such material proximal to the site of obstruction[7]

No doubt under most conditions the stimuli which promote the release of neurohypophysial hormones also stimulate the production of these substances in the hypothalamus to enable the discharge of hormone into the blood to continue and eventually to replenish the depleted stores At present the integration of secretion and release is incompletely understood and there is moreover no agreement as to the specificity of a given stimulus in terms of the hormone or hormones released For example some workers claim to have shown that a stimulus appropriate to the release of ADH may also evoke the discharge of oxytocin and vice versa[7] Final judgment on these matters must await further experimental studies

1 *ADH* There appears to be a constant basal secretion of ADH which is regulated by the osmotic pressure of the blood This secretory activity provides a reciprocal relationship between the neurohypophysis on the one hand and the kidneys sweat glands intestine and lungs on the other (Fig 45) The ingestion of water lowers the osmotic pressure of blood and inhibits the secretion and release of ADH while dehydration has the opposite effect There is good reason to believe that the end organs which respond to these changes in osmotic pressure (osmoreceptors) consist of small vesicles found within the cells of the supraoptic nucleus—cells which are closely related to the capillaries of this nucleus

Superimposed upon this background there exists a mechanism which alters the secretion and the release of ADH this is medicated through nervous reflexes which act upon the supraoptic nucleus In this way stressor stimuli (especially emotional stress but also physical stress) stimulate the production of ADH and hence depress the rate of excretion of water Many changes in the external environment make use of this mechanism thereby causing a decline in the volume of urinary output This response to stressor stimuli temporarily disturbs the basic level of ADH secretion (Fig 46)

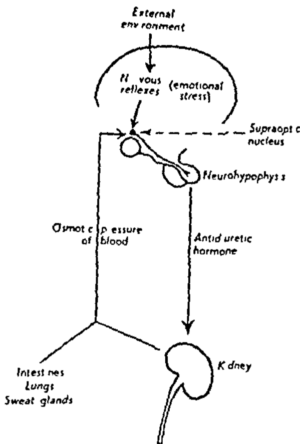


Fig 45 The control of the secretion of antidiuretic hormone The base line level of secretion is determined by the osmotic pressure of the blood to which the supraoptic nucleus is sensitive Stimuli from the environment affect the secretion of ADH either by nervous reflex paths or by inducing a state of emotional stress (*G W Harris*)

Control

Since the function of the neurohypophysis is to release the two hormones ADH and oxytocin into the blood stream the factors controlling neurohypophysial function must be regarded as being of two types—those which govern the rate of secretion of these hormones and those which control the rate of their release into the blood So far the distinction between these two controlling mechanisms has been difficult to preserve in experimental procedures but such a distinction should not be overlooked in considering neurohypophysial function

The activity of the neurohypophysis is regulated by the hypothalamus and Harris has pointed out that this relationship is as constant and as intimate as that which exists between a voluntary muscle and the lower motor neurones which supply it For if the hypothalamo hypophysial nerve tracts are severed the neurohypophysis ceases to function and undergoes atrophy[6] Experimental obstruction to the passage of neurosecretory material along these tracts by means of some form of pressure causes an accumulation of such material proximal to the site of obstruction

No doubt under most conditions the stimuli which promote the release of neurohypophysial hormones also stimulate the production of these substances in the hypothalamus to enable the discharge of hormone into the blood to continue and eventually to replenish the depleted stores At present the integration of secretion and release is incompletely understood and there is moreover no agreement as to the specificity of a given stimulus in terms of the hormone or hormones released For example some workers claim to have shown that a stimulus appropriate to the release of ADH may also evoke the discharge of oxytocin and vice versa[7] Final judgment on these matters must await further experimental studies

1 *ADH* There appears to be a constant basal secretion of ADH which is regulated by the osmotic pressure of the blood This secretory activity provides a reciprocal relationship between the neurohypophysis on the one hand and the kidneys sweat glands intestine and lungs on the other (Fig 45) The ingestion of water lowers the osmotic pressure of blood and inhibits the secretion and release of ADH while dehydration has the opposite effect There is good reason to believe that the end organs which respond to these changes in osmotic pressure (osmoreceptors) consist of small vesicles found within the cells of the supraoptic nucleus—cells which are closely related to the capillaries of this nucleus

Superimposed upon this background there exists a mechanism which alters the secretion and the release of ADH this is mediated through nervous reflexes which act upon the supraoptic nucleus In this way stressor stimuli (especially emotional stress but also physical stress) stimulate the production of ADH and hence depress the rate of excretion of water Many changes in the external environment make use of this mechanism thereby causing a decline in the volume of urinary output This response to stressor stimuli temporarily disturbs the basic level of ADH secretion (Fig 46)

Adrenaline appears to inhibit the release of ADH without interfering with its action upon the kidney There is good evidence to show that at least some of the afferent nerves which affect the function of the supraoptic nucleus are cholinergic in nature[4]

The volume and composition of extracellular fluid also appear to affect the release of ADH from the neurohypophysis It has been shown that a fall in extracellular fluid volume or a rise in its osmotic pressure can promote the release of ADH

Miscellaneous factors affecting the release of antidiuretic hormones include

(i) Increase in circulating blood volume may cause a fall in the release of ADH It is thought that this effect is due to reflex stimulation of stretch receptors in the heart pulmonary vessels and great veins A fall in blood volume has the opposite effect

(ii) Nicotine acetylcholine morphine barbiturates ether and chloroform increase the rate of release of ADH while alcohol exerts the opposite effect Nicotine (and smoking) constitutes one of the most powerful anti diuretic stimuli known

(iii) Exposure to cold suppresses the rate of release of ADH

(iv) Hypnosis can bring about a fall in the rate of release of the hormone

(v) A decrease in extracellular fluid volume may stimulate the release of ADH

(vi) Pain and emotion increase the rate of release of ADH

2 *OXYTOCIN*[4] Evidence for a constant basal secretion of oxytocin is less readily demonstrated than in the case of ADH and the possibility that stimuli which promote the secretion of ADH may also affect oxytocin secretion makes the control of this hormone complex However there are three conditions under which the release of oxytocin from the neurohypophysis appears to be important (Fig 40)

1 During the later phases of pregnancy distension of the uterus excites nervous pathways in the spinal cord which convey impulses to the hypothalamus and so lead to the secretion of oxytocin by the neurohypophysis This secretion produces forceful uterine con tractions which may be important in the initiation of labour

2 During coitus a similar reflex excitation of oxytocin secretion has been demonstrated in certain species Oxytocin then causes

increase in uterine motility and this assists the passage of sperm along the female genital tract Measurements of the pressure within the female tract and of the rate of movement of sperm within the genital tract suggest that such changes in uterine motility are important in the transport of the male germ cell

3 Lactation depends upon two separate processes—the secretion of milk which is controlled by the adenohypophysis and the discharge of milk which is controlled by a neurohumoral reflex The nervous stimulation of the nipple produced by suckling excites pathways in the spinal cord which convey impulses to the hypothalamus This in turn promotes the secretion of oxytocin by the neurohypophysis and this hormone causes the contraction of the myoepithelial tissue of the mammary gland which produces milk-ejection (Fig 40) Emotional stress interrupts the mechanism of milk-ejection as a result of the action of adrenaline which is released from the adrenal medulla during exposure to stress or stimuli Adrenaline acts centrally by preventing the secretion of oxytocin

It is commonly accepted that the sensitivity of the myometrium to oxytocin varies with changes in sex hormone secretion in an apparently purposeful manner—being increased under the influence of oestrogens and decreased by progesterone During pregnancy the cervix responds to a greater degree than the body (favouring retention) and the reverse is true in the later stages of pregnancy especially shortly before delivery when the response favours expulsion of the uterine contents Again in the non pregnant uterus[7] the cervix shows a greater response while under the influence of oestrogens (favouring retention of sperm) than under that of progesterone

THE ROLE OF THE ENDOCRINE GLANDS IN WATER BALANCE

The total volume of fluid within the various compartments of the body is the result of a balance between the intake and output of water Fluid intake is regulated by thirst and fluid absorption is largely the result of physico chemical factors operating in the intestinal mucosa

FLUID OUTPUT Water is eliminated from the body by the kidneys sweat glands skin surface lungs alimentary canal and various exocrine glands (salivary lachrimal etc) The kidney is primarily concerned with body fluid regulation whereas loss from

other tissues is incidental to the special functions which these fulfil

REGULATION OF RENAL WATER OUTPUT The regulation of the osmotic concentration and the volume of body fluids is a homeostatic process and is the result of neurohumoral mechanisms which promote the elimination or conservation of water and sodium by the kidneys The formation of urine is the result of three processes glomerular filtration tubular reabsorption and tubular secretion The elimination of water is the result of the difference between glomerular filtration and tubular reabsorption Reabsorption of water consists of two processes (i) passive or obligatory reabsorption which is an osmotic phenomenon and (ii) active or facultative reabsorption which is controlled by the antidiuretic hormone in this way the kidney can make rapid adjustments of water excretion to suit the needs of the moment

THE EFFECT OF HORMONES UPON FACULTATIVE REABSORPTION OF WATER

(1) *Antidiuretic hormone* As mentioned above antidiuretic hormone does not affect glomerular filtration but increases the rate of reabsorption of water by the epithelium of the renal tubules It has been shown that ADH causes influx of water through the skin of amphibia by causing dilation of minute pores These pores are normally scarcely large enough to admit water but can be dilated in such a way as to alter the permeability of the skin to water These observations have been applied to the mammalian kidney by some workers who believe that the hormone affects the renal tubules in the same way namely by causing dilation of microscopic pores to allow a passive transfer of water

Wirz[7] has shown that the fluid in the proximal convoluted tubule is always isotonic while that within the distal convoluted tubule is hypotonic when the kidney is producing dilute urine but when the kidney is concentrating the fluid in the distal convoluted tubule reaches isotonicity before leaving this part of the nephron The fluid of the distal tubule is therefore never hypertonic so that when hypertonic urine is being produced some mechanism must exist for concentrating the fluid in the collecting ducts There is good evidence in some species of animal to show that the tip of the renal papilla is a region of hypertonicity and Wirz believes that the loop of Henle comes to contain hypertonic fluid as it approaches the tip of the papilla When the kidney is concentrating equilibrium is established between this hypertonic

fluid and that of the collecting ducts as these traverse the papilla Wirz[7] further suggests that in the absence of antidiuretic hormone the walls of the distal convoluted tubule and those of the collecting ducts behave as though they were impermeable to water On the other hand under the influence of ADH pores in the walls

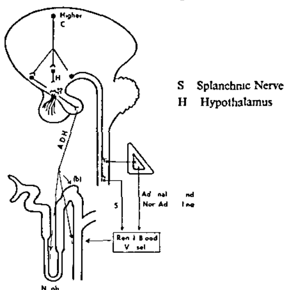


Fig 46 (*a*) Neurohumoral mechanisms by which such conditions as emotion exercise and pain result in diminished urinary output The three parts of the nephron from which ADH promotes the reabsorption of water are shown (*a*) the descending limb of the loop (*b*) the distal convoluted tubule (*c*) the collecting tubules

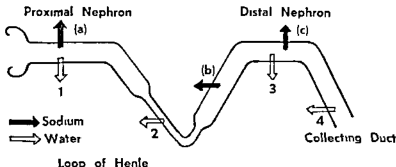


Fig 46 (*b*) Diagram showing the sites at which reabsorption of sodium and water take place in the nephron and collecting duct (*After Wir*) ADH controls the reabsorption of water at 2 3 and 4 Reabsorption of sodium and water at 1 is controlled by osmotic pressure

of the collecting ducts are allowed to open and equilibration takes place between the hypertonic fluid in the loop and the fluid in the collecting ducts As a result of this equilibration the collecting system comes to contain hypertonic fluid which has reached the concentration of the definitive urine Meanwhile the fluid in the loop enters the ascending limb and leaves the hypertonic region at the tip of the papilla As it does so reabsorption of sodium takes place which causes the fluid entering the distal convoluted tubule to become hypotonic (Fig 46)

It remains to explain the mechanism by means of which the fluid in the loop of Henle becomes hypertonic The sodium which is absorbed along the ascending limb of the loop renders the extracellular fluid in the surrounding tissue hypertonic Under the influence of ADH the fluid in the descending limb is permitted to equilibrate with its hypertonic surroundings by the opening of microscopic pores in the wall of this part of the nephron

There are therefore four sites of water reabsorption in the kidney (Fig 46) Firstly water is reabsorbed in the proximal convoluted tubule as the result of an obligatory and passive process which removes approximately seven-eighths of the filtered water Secondly water is reabsorbed in the descending limb and this leads to the production of a hypertonic fluid within the tip of the loop Thirdly water is absorbed from the distal convoluted tubule in order to allow water reabsorption to catch up with the reabsorption of sodium which has taken place in the ascending limb of the loop (Fig 46) Finally water is reabsorbed in the concentrating kidney from the collecting ducts by equilibration with the hypertonic fluid at the tip of the loop

The reabsorption of water from the last three of these sites is believed to be controlled by antidiuretic hormone In the absence of this hormone the distal nephron and the collecting system behave as though they were impermeable to water but in the concentrating kidney *ADH brings about the opening of pores which allows reabsorption of water to take place at these three sites* While this theory has the virtue that it requires only one mechanism for the action of ADH—a mechanism by means of which the hormone is believed to act on the skin of amphibia the conclusions which have been drawn from the experiments of Wirz have yet to gain universal acceptance

(2) *Glucocorticoids* Glucocorticoids assist the body to remove a water load (e g after the ingestion of large volumes of water) while mineralocorticoids appear to promote the reabsorption of

water (together with sodium) from the renal tubules (see Chapter II) The mechanism by which glucocorticoids affect the excretion of water is not clear but they appear to suppress the release of ADH from the neurohypophysis and the inactivation of this hormone by the tissues appears to depend to some extent upon the presence of glucocorticoids[7]

(3) *Catecholamines* Adrenaline and nor adrenaline produce a variable effect upon the excretion of water Under certain experimental conditions they produce diuresis under others they cause antidiuresis The mechanism of these effects is probably complex and may depend in part upon vascular changes which affect glomerular filtration and upon the release of ADH from the neurohypophysis

DIABETES INSIPIDUS

Destruction of the supraoptic and paraventricular nuclei or the removal of the neurohypophysis (or its destruction by morbid processes) results in the passage of large volumes of urine This condition is called diabetes insipidus and represents failure of ADH secretion The only abnormality which follows this failure is the inability of the body to conserve water The passage of large volumes of dilute urine is accompanied by an insatiable thirst The daily output of urine usually exceeds 5 litres and may be twice this amount The specific gravity of such urine is always low (usually below 1 008)

For the development of the full picture of diabetes insipidus the adenohypophysis must be functioning normally The reason for this is not certain but growth hormone and TSH seem to be the important factors responsible for the behaviour of the adenohypophysis under these circumstances Recent work however suggests that changes in the vascular condition of the kidney are also important in explaining this phenomenon[8]

REFERENCES

The control of neurohypophysial activity is described in detail in the Neural Control of the Pituitary Gland by G W Harris Edward Arnold London 1955

1 Maximow A A and Bloom W A Textbook of Histology p 308 Saunders Philadelphia 1943
2 Du Vigneaud et al J Amer Chem Soc 75 4880 1953
3 Du Vigneaud et al J Amer Chem Soc 75 4879 1953
4 Bargmann W and Scharrer E Amer Scientist 39 255 1951

5 Richter C P Proc of the Assoc for Res in Neur and Ment Dis Pituitary Gland Williams and Wilkins Co Baltimore 1938
6 Harris G W Neural Control of the Pituitary Gland Edward Arnold London 1955
7 Heller H The Neurohypophysis Butterworth London 1957
8 White H L et al Amer J Physiol 136 584 1942
9 De Langen C D Acta Med Scand CLX (fasc iv) 275 1958

CHAPTER IX

THE ADRENAL MEDULLA

Introduction

The adrenal medulla is intimately connected with the nervous system and develops from the neural crest The cells of the medulla are in fact modified ganglion cells which remain in intimate contact with preganglionic fibres of the sympathetic nervous system and their secretory activity seems to depend to a great extent upon the stimulation which they receive from these nerves these facts can be illustrated experimentally Section of the splanchnic nerve or painting the adrenal gland itself with nicotine will prevent the medulla from secreting on the other hand stimulation of the splanchnic nerve produces an increase in medullary secretion Through the splanchnic nerves the medulla is connected with centres in the posterior hypothalamus

The adrenal medulla is not essential to life but its activity is greatly increased during states of emergency For example in those who are about to perform some strenuous exertion the mere thought of this physical effort is sufficient to stimulate the secretory activity of the medulla Laboratory animals in which the medulla has been destroyed show surprisingly little change in behaviour in the protected environment of the laboratory

Embryology

The medulla is derived from the ectodermal tissue which gives rise to the cells of the sympathetic ganglia During the seventh week of intrauterine life when the cortex is already prominent masses of these cells invade the medial side of the primitive adrenal cortex and eventually gain a central position where they develop together with a profuse network of sinusoidal capillaries

Histology

The boundary between cortex and medulla is for the most part irregular The majority of the medullary cells contain fine brown granules which because they stain with chromates are called

chromaffin cells Less numerous are the sympathetic ganglion cells which occur singly or in groups The axons of the ganglion cells end around the chromaffin cells

Anatomy

In addition to the chromaffin tissue in the medulla the same tissue exists outside the adrenal gland in the following sites

1) Paraganglia which are small masses (about 3 mm in diameter) within or alongside the capsules of sympathetic ganglia

2) Along the abdominal aorta above the origin of the inferior mesenteric artery similar paraganglia are to be found

3) The organs of Zuckerkandl which are small masses of chromaffin tissue situated on either side of the origin of the inferior mesenteric artery

4) In the carotid glands at the point of bifurcation of the common carotid artery

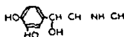
5) Within the liver and the heart

Function

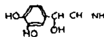
The function of the adrenal medulla is to secrete two hormones namely adrenaline and nor adrenaline also called epinephrine and nor epinephrine No other function of the medulla is known beyond the secretion of these two hormones in varying quantities under varying conditions It is possible that the gland secretes a third hormone called isoprenaline

Chemistry

Adrenaline and nor adrenaline are amines



Adrenaline



Nor adrenaline

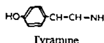
The prefix nor is derived from the German Nitrogen ohne Radikal the radical referring to the CH_3 group of adrenaline Adrenaline and nor adrenaline are together referred to as catecholamines From the structural formulae of the two hormones it will be seen that adrenaline has an additional methyl group which changes the side chain from a primary amine in the case of nor

adrenaline –C–C–NH to a secondary amine in adrenaline

$-\overset{|}{\underset{|}{C}}-HN-\overset{|}{\underset{|}{C}}-$ (page 4) The medulla contains ascorbic acid which may play some part in protecting these amines from oxidation

Synthesis

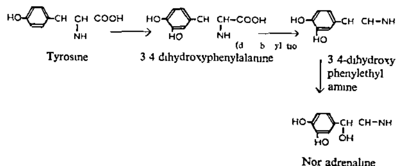
The structure of adrenaline resembles that of the amino acids tyrosine and phenylalanine which suggests that these acids may be the source of the hormone in the body Moreover it is a relatively simple matter to convert tyrosine to adrenaline in a test tube However in vivo experiments have failed to show that the adrenal medulla is capable of converting either tyrosine or phenylalanine into adrenaline The synthesis of adrenaline remains therefore somewhat obscure although recent experiments have suggested a number of possible solutions to this problem For example tyrosine can be converted by decarboxylation (loss of CO) in the kidney to tyramine Tyramine when perfused through the isolated adrenal gland results in the production of a substance which shows all the physiological properties of adrenaline



Tyramine

Again it is known that mammalian liver is capable of converting phenylalanine to tyrosine this suggests that phenylalanine may be involved in the synthesis of adrenaline and studies employing isotopically labelled phenylalanine support this possibility

The following alternative pathway has received most support within recent years —



Tyrosine

3 4 dihydroxyphenylalanine

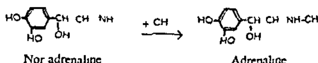
3 4-dihydroxy phenylethyl amine

Nor adrenaline

An enzyme has been found in mammalian liver which is capable of converting 3 4-dihydroxyphenylalanine (DOPA) to 3 4 dihydroxyphenylethylamine (DOPAMINE) this reaction is an example of decarboxylation and the enzyme system involved includes pyridoxal phosphate

Several links in the chain of evidence required to establish this method of synthesis are missing for example DOPA has not been found in the adrenal medulla nor has the enzyme pyridoxal phosphate

Nor adrenaline is converted to adrenaline by the process of transmethylation (page 5) during which the amino acid methionine donates a methyl group —



It is important not to regard this last reaction as indicating that nor adrenaline is merely a precursor of adrenaline

The relative proportions of adrenaline and nor adrenaline in the medulla varies from species to species but in man adrenaline comprises about 90 per cent of the catecholamines in the gland In other tissues the amount of nor adrenaline present is correlated with the extent of their adrenergic innervation

In the case of both adrenaline and nor adrenaline the laevo ( ) isomer is the active form

Histology in Relation to Function

As with other endocrine glands the secretion of two hormones by the medulla raises the question of whether each has its own specific parent cell or whether both are secreted by one type of cell In the case of the medulla there are champions of either view[4 5] A good deal of evidence favours the view that one type of cell produces adrenaline and another produces nor adrenaline This evidence is chiefly of three kinds —

(i) Stimulation of different parts of the hypothalamus releases different proportions of the two hormones into the blood of the suprarenal vein

(ii) Certain reflex stimuli act selectively e g carotid occlusion favours nor adrenaline secretion while sciatic nerve stimulation produces a mixture rich in adrenaline

(iii) Recent histochemical studies support the conception of two secreting cell types within the medulla

The available evidence is not conclusive but at the present time appears to favour the view that in the medulla there are to be found two types of cell each of which produces only one hormone In any case the cells which secrete adrenaline are thought to produce this hormone from nor adrenaline

Actions of Adrenaline and Nor-Adrenaline

Adrenaline and nor adrenaline exert their most striking effects upon the contractility of cardiac and smooth muscle In addition they affect the rate of certain reactions involved in the metabolism of carbohydrates

Adrenaline

CIRCULATION It should be clearly understood that the effects of adrenaline and nor adrenaline upon the circulation are greatly influenced by the conditions of the experiment e g dose route of administration the species anaesthetic used and by certain compensatory reflex adjustments

In addition it has been pointed out that peripheral resistance in the circulation is determined by the sum of the resistance offered by the arterioles which allow blood to enter the capillaries on the one hand and that offered by the arterio venous communications which allow the blood to by pass the capillaries and flow direct from arteriole to venule on the other hand The diastolic blood pressure is determined by the balance between these two flows For example the capillary microscope has revealed that under the influence of nor adrenaline some capillary loops disappear while others become narrower (especially on the arterial side of the loop) and yet the diastolic pressure remains unaltered or shows a slight fall Since the capillaries can be seen to close it must be assumed that the blood can flow along a different route and so by pass the capillary loops in this way the peripheral resistance (and hence the diastolic pressure) remains essentially unchanged [4]

(a) *Heart* Adrenaline produces tachycardia and increases the irritability and contractility of heart muscle In large doses it may produce ventricular extrasystoles and eventually ventricular fibrillation These effects result from the direct action of adrenaline on heart muscle In addition the hormone stimulates the atrioventricular node and shortens A V conduction time when this is prolonged by vagal stimulation

(b) *Cardiac output* As a result of these effects upon the heart adrenaline increases the cardiac output and raises the systolic blood pressure while the diastolic pressure remains normal or shows a slight fall

(c) *Blood vessels* In general adrenaline promotes contraction of the smooth muscle of arterioles However the effects observed experimentally when adrenaline is injected or applied locally to blood vessels are not always consistent It will be seen that adrenaline differs in this respect from nor adrenaline which is always constrictor in its effect upon blood vessels except those of the coronary circulation The important site of action of catecholamines is on the precapillary sphincter which causes diversion of blood into venules thereby short-circuiting blood away from the capillaries Adrenaline causes intense vasoconstriction of the arterioles and capillaries of the skin and mucous membranes It also constricts the arterioles and capillaries of the kidney and of those tissues supplied by the splanchnic nerves In the case of skeletal muscle however the action of adrenaline is more complex Small doses produce a transient dilator effect[10] large and repeated doses may cause constriction but adrenaline usually causes an increase in blood flow through the limbs Adrenaline causes vasodilation of the coronary cerebral and hepatic vessels and an increase in pressure in the pulmonary arteries (Table I) The result of these various responses is a fall in total peripheral resistance that is a net vasodilator action (Table I) since in the normal subject the blood flow through the liver kidneys skeletal muscle and brain accounts for the greater part of the cardiac output

TABLE I

Tissue	Flow before injection of Adrenaline or Nor adrenaline ml /min	Flow (ml /min) during administration of			
		Adrenaline		Nor adrenaline	
			% Change		% Change
Liver	1 500	3 000	+100	1 500	0
Kidneys	1 500	900	− 40	1 200	−20
Skeletal Muscle	1 000	2 000	+100	1 000	0
Brain	750	900	+ 20	675	−10
TOTAL	4 750	6 800	+ 40	4 375	−8

These changes in vasomotor tone explain the effect of adrenaline on the blood pressure in the following manner —

(i) The rise in systolic pressure is due to increased cardiac output which in turn follows increased force of ventricular con traction and an increase in venous return

(ii) The slight fall in diastolic pressure results from the overall decrease in peripheral resistance

2 *RESPIRATORY SYSTEM* Adrenaline brings about relaxation of the smooth muscle of bronchi and after a period of apnoea causes an increase in the rate and depth of respiration This effect upon the rate and depth of respiration is due to the action of adrenaline upon the nervous pathways which control respiration but the exact point of action is unknown

3 *METABOLISM* (a) *Carbohydrate metabolism* Adrenaline produces an increase in blood sugar by stimulating the con version of glycogen to glucose in the liver In addition the hor mone promotes the oxidation of tissue glycogen to lactic acid which is converted to glycogen in the liver with the result that liver glycogen actually increases as a result of the action of adrena line In muscle for example adrenaline stimulates the conversion of glycogen to lactic acid by increasing the local concentration of phosphorylase an enzyme which promotes the production of hexose phosphates Some of the lactic acid formed in muscle diffuses into the blood stream from which it is removed by the liver and converted to glycogen In this way muscle glycogen indirectly contributes to the increase in blood sugar brought about by the action of adrenaline (Fig 53)

Another factor which plays a part in the production of hyper glycaemia by adrenaline is a decline in the rate at which the tissues use glucose Glucose tolerance is reduced under the influence of adrenaline and the arterio venous glucose difference is small even when the arterial blood glucose level is high In normal animals exposure to emotional and physical stress induces a temporary hyperglycaemia due to the release of adrenaline In some species when doses of adrenaline too small to cause hypertension are injected hyperglycaemia is observed One of the few abnormal responses shown by animals in which the adrenal medulla has been destroyed is a delay in spontaneous recovery from hypo glycaemia

(b) *General metabolism* Adrenaline causes increase in the oxygen consumption and in basal metabolic rate This effect is not mediated through the thyroid gland but does not occur if the

liver has been removed The hormone also causes a rise in serum potassium

4 *SKIN* Adrenaline depresses the action of sweat glands and brings about a pilomotor response

5 *CENTRAL NERVOUS SYSTEM* Injections of adrenaline administered to healthy individuals cause anxiety, apprehension restlessness and a sense of fatigue in the back and legs together with a coarse tremor Within 10 seconds of an injection of adrenaline an increase in the rate and depth of respiration is noticed and it becomes impossible to hold a moderate inspiration for more than 15 seconds About 10 seconds after the changes in respiration the heart rate begins to increase but palpitations are not usually experienced before a further 30 seconds have elapsed Soon after the subjective effects upon the heart a sense of fatigue and a coarse tremor are noticed

6 *ENDOCRINE GLANDS* The action of adrenaline upon other endocrine glands has been discussed elsewhere Here these actions will be briefly summarised

Adrenaline causes —

- (i) increased production of ACTH by the adenohypophysis
- (ii) decreased production of TSH by the adenohypophysis
- (iii) decrease in the rate of release of ADH from the neurohypophysis

7 *EYE* Adrenaline causes pupillary dilation protrusion of the eyeball and retraction of the upper eyelid

8 *GASTROINTESTINAL TRACT* The hormone causes relaxation of the smooth muscle of the alimentary tract together with stimulation of the sphincters

9 *GENITO URINARY ORGANS* Adrenaline stimulates the muscle of the ureter and the sphincter of the bladder, the retractor penis and the body of the pregnant uterus It causes relaxation of the wall of the bladder

10 *MISCELLANEOUS ACTIONS*

- (a) Increase in the coagulability of blood
- (b) Increase in the secretion of saliva
- (c) Contraction of the smooth muscle of the splenic capsule The importance of this action in bringing about an increase in blood volume has probably been exaggerated

(d) Both adrenaline and nor adrenaline antagonise the action of MSH upon the melanocytes of the skin (page 159).

Nor Adrenaline

It will be seen from Table II that nor adrenaline and adrenaline for the most part act in the same direction. When they are excitors the activity of the two compounds is of the same order (adrenaline usually being somewhat more potent) but when they inhibit adrenaline is usually (but not always) more active than nor adrenaline. Generally speaking both stimulate the vascular system and inhibit the viscera. The chief difference between the two hormones lies in the intense peripheral vasoconstrictor action of nor adrenaline on the one hand and the increase in cardiac output brought about by adrenaline on the other.

1 *CIRCULATION* (a) *Heart* Nor adrenaline causes an increase in the irritability and contractility of heart muscle but it causes only a slight rise (or even a fall) in heart rate while the cardiac output remains unchanged or shows a slight fall[10]. The decrease in heart rate is due to a reflex increase in vagal tone (from carotid sinus and aortic receptors) brought about by the associated rise in blood pressure.

(b) *Blood vessels* Nor adrenaline exerts its greatest effect upon blood vessels. It causes marked vasoconstriction a considerable increase in peripheral resistance together with a sharp rise in systolic and diastolic blood pressures. In addition nor adrenaline causes a rise in pulmonary blood pressure[0]. It does not affect the blood flow through liver and muscle but causes a slight fall in that through the kidney and brain (Table I).

The overall action of nor adrenaline is therefore vasoconstrictor resulting in a rise in total peripheral resistance. Table I gives some quantitative data concerning the effect of adrenaline and nor adrenaline upon the blood flow through various organs.

These observations make it possible to explain the effect of nor adrenaline upon the blood pressure.

(i) The rise in systolic blood pressure is due to increased force of ventricular contraction and to an increase in peripheral resistance.

(ii) The rise in diastolic pressure is due to the increase in total peripheral resistance.

There is some experimental evidence to suggest that nor adrenaline produces an increase in venous tone and in this way

liver has been removed The hormone also causes a rise in serum potassium

4 *SKIN* Adrenaline depresses the action of sweat glands and brings about a pilomotor response

5 *CENTRAL NERVOUS SYSTEM* Injections of adrenaline administered to healthy individuals cause anxiety, apprehension restlessness and a sense of fatigue in the back and legs together with a coarse tremor Within 10 seconds of an injection of adrenaline an increase in the rate and depth of respiration is noticed and it becomes impossible to hold a moderate inspiration for more than 15 seconds About 10 seconds after the changes in respiration the heart rate begins to increase but palpitations are not usually experienced before a further 30 seconds have elapsed Soon after the subjective effects upon the heart a sense of fatigue and a coarse tremor are noticed

6 *ENDOCRINE GLANDS* The action of adrenaline upon other endocrine glands has been discussed elsewhere Here these actions will be briefly summarised

Adrenaline causes —

(i) increased production of ACTH by the adenohypophysis

(ii) decreased production of TSH by the adenohypophysis

(iii) decrease in the rate of release of ADH from the neurohypophysis

7 *EYE* Adrenaline causes pupillary dilation protrusion of the eyeball and retraction of the upper eyelid

8 *GASTROINTESTINAL TRACT* The hormone causes relaxation of the smooth muscle of the alimentary tract together with stimulation of the sphincters

9 *GENITO URINARY ORGANS* Adrenaline stimulates the muscle of the ureter and the sphincter of the bladder the retractor penis and the body of the pregnant uterus It causes relaxation of the wall of the bladder

10 *MISCELLANEOUS ACTIONS*

(a) Increase in the coagulability of blood

(b) Increase in the secretion of saliva

(c) Contraction of the smooth muscle of the splenic capsule The importance of this action in bringing about an increase in blood volume has probably been exaggerated

Comparison Between the Effects of Adrenaline and Nor Adrenaline

1 *CIRCULATION* Adrenaline and nor adrenaline superficially resemble one another in their actions upon the circulation because they both increase the systolic and mean arterial pressures The hypertensive effect of nor adrenaline is due to an increase in peripheral resistance while that of adrenaline results from an increase in cardiac output in spite of a fall in total peripheral resistance Muscle blood flow is increased by adrenaline and is unaltered or decreased by nor adrenaline Splanchnic blood flow is decreased both by adrenaline and nor adrenaline Both hormones cause a decrease in renal blood flow Adrenaline and nor adrenaline cause a decrease in blood flow in the skin The algebraic sums of these blood flow changes indicate a fall in total peripheral resistance in the case of adrenaline and a rise in the case of nor adrenaline

The action of catecholamines upon blood vessels is a direct one and to explain the different actions of adrenaline and noradrenaline upon different vessels some workers have suggested the presence of two receptors (α and β adrenotrophic receptors) within the tissues Mixtures of adrenaline and nor adrenaline cause vasodilatation in skeletal muscles unless the concentration of nor adrenaline in the mixture exceeds 90 per cent

2 *OTHER ACTIONS* Adrenaline exerts a much stronger effect upon carbohydrate metabolism oxygen consumption central nervous system and adenohypophysial function than nor adrenaline

TABLE III

Circulatory changes produced by adrenaline and nor adrenaline

Compound	*Cardiac Output*	*Systemic Blood Pressure*			*Mean Pulmonary Pressure*	*Total Peripheral Resistance*	*Pulse Rate*
		Systolic	*Diastolic*	*Mean*			
Adrenaline	+++	+++	unchanged	+	++	-	+
Nor Adrenaline	unchanged	+++	++	++	++	+++	-

+ indicates an increase and – indicates a decrease

The difference between the action of adrenaline and that of nor adrenaline together with the proposed secretion of each hormone by a separate cell type suggest that each plays a distinct

promotes venous return of blood to the heart This may explain in part the beneficial effect of the hormone in the treatment of shock

2 *SMOOTH MUSCLE* The action of nor adrenaline upon smooth muscle like that of adrenaline may be one of excitation or of inhibition depending upon the organ, or in some instances upon its physiological state[9] In general the two hormones exert the same qualitative effects upon smooth muscle and Table II gives some information about the quantitative differences between these effects

3 *CARBOHYDRATE METABOLISM* Nor adrenaline exerts the same effect upon carbohydrate metabolism as adrenaline but it is only about one quarter as potent

4 *CENTRAL NERVOUS SYSTEM* Nor adrenaline does not produce anxiety fear or palpitations Normal subjects are often unaware that the hormone has been injected

TABLE II

Comparison of the physiological activity of adrenaline and nor adrenaline

System	Function	*Effect*		Relative Activity A/N
		Adrenaline	*Nor adrenaline*	
Vascular	B P Systolic	Raised	Raised	0 5
	B P Diastolic	None	Raised	
	Blood Vessels in Denervated Limb	Dilatation	Constriction	
	Peripheral Resistance	Decreased	Increased	
Heart	Heart Rate	Increased	Slightly increased	20
	Cardiac Output	Increased	Unchanged	
	Coronary Vessels	Dilatation	Dilatation	1
Respiratory	Bronchial Muscle	Inhibition	Inhibition	20
Carbohydrate Metabolism	Blood Sugar	Raised	Raised	4
Eye	Pupillary Dilators	Stimulation	Stimulation	15
Alimentary	Small Intestine	Inhibition	Inhibition	2
	Large Intestine	Inhibition	Inhibition	1
Genital	Uterine Muscle	Inhibition	Inhibition	100

vous tissues) may inactivate catecholamines by the process of oxidative deamination

(ii) *Inactivation of hydroxyl groups* The OH groups present in the molecules of adrenaline and nor adrenaline are essential to their physiological activity These groups could be inactivated by esterification (page 4) or by oxidation Esterification with sul phuric acid can occur in the intestine but is probably not import ant in blood and other tissues Oxidation by catecholoxidase has been suggested as an alternative means of inactivation of the two hormones Nor adrenaline appears to be inactivated more readily than adrenaline and it seems likely that the methyl group of adrenaline protects this substance to some extent from inactivation by amine oxidase

Normal human urine contains a mixture of catecholamines of which some 15 per cent is adrenaline and the remainder is noradrenaline The average urinary output of adrenaline in 24 hours is between 3 and 9 micrograms and that of nor adrenaline between 15 and 45 micrograms[3] This probably constitutes only a very small fraction of the total amount of the two hormones secreted by the body since only 2 per cent of an infusion of nor adrenaline can be recovered from the urine The exact proportions in which the two hormones appear in normal human urine however is subject to considerable variation

Control of Medullary Function

The adrenal medulla is not essential to life To what extent this observation can be explained by the activity of extramedullary chromaffin tissue is uncertain Adrenergic nerves can continue to secrete nor adrenaline in the absence of medullary tissue and it would appear that the functions of adrenaline are to a large extent dispensable Of the three major functions of the catechol amines—maintenance of vasomotor tone stabilisation of carbohy drate metabolism and response of the body to stress the first is performed by nor adrenaline from adrenergic nerves and in the absence of medullary tissue the other two can be assumed by a number of compensatory mechanisms some of which involve the adrenal cortex

The cells of the adrenal medulla are in intimate contact with the cells of the sympathetic nervous system and their secretory activity is to a large extent dependent upon stimulation through these nervous pathways[14] Centres in the posterior hypothalamus appear to control the activity of the medulla by way of the splan

role in the body Moreover it seems highly probable that either hormone can be secreted independently of the other Nor adrenaline is almost exclusively the mediator of adrenergic nerve impulses and seems well suited to making rapid circulatory adjustments to maintain the blood pressure whereas adrenaline is suited to making adjustments of metabolic functions (especially in checking hypoglycaemia) and in aiding the redistribution of blood to such active tissues as skeletal muscle Both hormones are important *in the responses of the body to stress but the sympathetic* nervous system is especially concerned with the regulation of the circulation while the adrenal medulla (which in man secretes more adrenaline than nor adrenaline) is essentially concerned with the protection of the body against stressor stimuli

Isoprenaline

A third substance has recently been isolated from the adrenal glands of several species including man [1 2] This substance is isopropyl nor adrenaline called isoprenaline Instead of the transmethylation which converts nor adrenaline to adrenaline addition of an isopropyl group $\begin{matrix} CH \\ \quad CH \\ CH \end{matrix}$ takes place Isoprenaline is about 10 times as potent as adrenaline in its bronchodilator action the significance of this third medullary product in the function of the gland remains uncertain

Metabolism

Both adrenaline and nor adrenaline are rapidly inactivated in vivo and in vitro Adrenaline is especially unstable in buffered solutions but its susceptibility to oxidation can be diminished in the presence of other reducing agents such as ascorbic acid and certain amino acids For this reason adrenaline is more stable in blood and in certain tissues than in water ascorbic acid serves to protect the hormone in the adrenal gland Adrenaline is oxidised *in vitro to a dark melanin like product* but the result of in vivo oxidation remains uncertain Some adrenaline is excreted in conjugated form as the sulphate and the glucuronide in which forms it appears in urine

Among the possible mechanisms of inactivation of catecholamines in vivo two have received most support —

(1) *Amine oxidase* It is possible that the enzyme amine oxidase (*which is widely distributed in glandular* muscular and ner

rate of secretion of nor adrenaline are believed to occur under conditions which threaten the stability of blood pressure levels

Assay

ADRENALINE[16] Assay of adrenaline is a difficult procedure and the small concentrations present in most biological specimens require the use of delicate biological methods Bioassay may be performed by the effect of an unknown solution on the blood pressure of the cat or by the inhibition of the rhythmic activity of the isolated rabbit intestine The chemical assays most frequently employed involve some modification of Shaw s method which depends upon the reduction of arsenomolybdic acid by adrenaline with the formation of a blue compound Fluorimetric assay depends upon conversion of catecholamines to the fluorescent substances adrenolutine and nor adrenolutine Newer chromatographic methods which give promise of very accurate results are at present under trial

NOR ADRENALINE[16] Nor adrenaline can be estimated by its stimulating effect upon the nictitating membrane of the cat or by a number of chemical methods

DISEASE OF THE ADRENAL MEDULLA

The clinical picture of excess of adrenaline and nor adrenaline secretion is seen in the rare tumours of the chromaffin cells of the medulla Such tumours produce hypertension and tachycardia together with a series of signs and symptoms attributable to excess of catecholamines The hypertension may be persistent or paroxysmal but in some cases it is absent Certain tests have been employed to assist in the diagnosis of such chromaffin tumours

A *PHARMACOLOGICAL TESTS*

1 *Adrenergic blocking agents* Dibenamine and benzodioxane (or piperoxan) are capable of destroying circulating adrenaline and nor adrenaline When these substances are injected into patients suffering from hypertension due to excessive secretion of catecholamines they cause a prompt fall in blood pressure in the case of patients suffering from hypertension due to other causes the blood pressure shows no significant change Apparently these substances more readily antagonise the circulating catecholamines than those produced by sympathetic adrenergic nerves so that doses which lower the hypertension produced by chromaffin tumours do not affect the blood pressure of patients suffering from

chnic nerves These observations are in accord with the role of the hypothalamus and that of the medulla in controlling the responses of the body to stressor stimuli (Fig 1)

In view of the dependence of the medulla upon nervous stimulation it is not surprising that the secretory activity of the gland shows great variation It is generally believed that under basal conditions the continuous stimulation resulting from nerve impulses arising from all over the body produces a basal level of secretion This basal level is stepped up by such moderate stimulation as that provided by walking

ADRENALINE Adrenaline is thought to be secreted at a basal rate by the adrenal medulla but in addition provision is made for a rapid increase in the secretion of adrenaline under conditions of emergency There are two chief stimuli which evoke this medullary response

(i) Hypoglycaemia

(ii) Exposure to stressor stimuli

This conception of an emergency secretion of adrenaline accords well with its widespread effects upon the body and was originally elaborated in the doctrine of Cannon[11] in which the belief was stated that fright flight fear and rage promote an immediate increase in the secretion and liberation of adrenaline by the medulla Haemorrhage exposure to cold asphyxia increased physical activity, excitation of the hypothalamus and stimulation of the sympathetic nerves to the endocrine glands are some of the factors which stimulate medullary activity Acetylcholine and histamine produce the same effect While it is undoubtedly true that noxious stimuli increase the rate of secretion of adrenaline and that many of its effects (such as stimulation of heart action and increase in blood flow through muscle) are useful responses to such stimuli it remains to be shown that the whole effect of adrenaline is necessary to adaptation to a changing environment[1] Much of the earlier work which sought to prove this failed to take into account the importance of the adrenal cortex while the work of Harris[13] indicates that adrenaline plays only a *minor role in the* control of adrenocortical activity under conditions of stress (Chapter XIII)

NOR ADRENALINE It appears that under normal conditions nor adrenaline is being constantly secreted by the sympathetic nerve endings and acts at the site of its liberation to maintain vasomotor tone and hence the blood pressure Variations in the

17 La Due J S et al Ann Int Med 29 914 1948
18 Emlet J R. et al J A M A 146 1383 1951
19 Gifford R Jr et al J.A M.A 149 1628 1952
20 Shapiro A P et al Am J Med 10 115 1951
21 Rolson J M and Keels C.A Recent Advances in Pharmacology Churchill London 1956
22 Von Euler Nor adrenaline Thomas Springfield 1957
23 Nickerson M Pharmacol Rev 1 27 1949
24 De Langen C D Acta Med Scand CLX (fasc iv) 271 1958

essential hypertension[23] The reason for this differential inhibition of catecholamines is uncertain

2 *Stimulants of chromaffin tissue secretion* Although histamine acetylcholine and quaternary ammonium bases do provoke a discharge of adrenaline from the normal medulla some medullary tumours are hypersensitive to these substances and small doses produce a striking hypertensive response This group of substances is useful in those cases of chromaffin tumours which do not show sustained hypertension The following substances are used in such tests —

(a) Histamine 0 05 mgm intravenously

(b) Tetraethylammonium chloride 200 mgm intravenously[17]

(c) Mecholyl 25 mgm subcutaneously

The exact mechanisms involved in chromaffin stimulation by these substances are uncertain The application and limitations of these tests have been reviewed by Shapiro et al[20]

B *URINARY EXCRETION OF ADRENALINE AND NOR ADRENALINE* Chemical and bioassay methods for estimating urinary levels of these hormones have proved useful in the diagnosis of medullary tumours and are more reliable than pharmacological tests[16]

REFERENCES

An excellent review of medullary function is given by M Goldenberg in Am J Med 10 627 1951 while reference number 22 of the following list gives the most exhaustive and recent treatment of the subject

1 Niklaeff M C Zeit. Exptl Med 42 213 1924
2 Schuler W and Wiedermann A Zeit Physiol Chem 233 235 1935
3 Goldenberg M Am J Med 10 627 1951
4 Hillarp N A and Hokfelt B Acta Physiol Scand 30 55 1953
5 Erlank O Endocrinol 57 363 1955
6 Peart W S J Physiol 108 491 1949
7 Holtz P and Schumann H J Naturwiss V 35 191 1948
8 Abel J J Bull J Hop Hosp 13 29 1902
9 Gaudino M and Levitt M F J Clin Invest 28 1487 1949
10 Swan H J C Brit Med J 1 1003 1952
11 Cannon W B Endocrin & Metab 2 171 1922
12 Bush I E J Endocrinol 9 95 1953
13 Harris G W Neural Control of the Pituitary Gland Edward Arnold London 1955
14 Dougherty T F and White A Amer J Anat 77 81 1945
15 Chen G and Wickel A Endocrinology 51 21 1952
16 Emmens G W Hormone Assay Academic Press Inc New York 1950

(a) Pyruvic acid
(b) Lactic acid
(c) Acetic acid

(iii) Certain less easily isolated intermediary compounds which include —

(a) Phosphoric acid esters of hexose sugars
(b) Phosphoric acid esters of triose sugars
(c) Four dicarboxylic acids —
Succinic acid
Fumaric acid
Malic acid
Oxaloacetic acid
(d) Five—and six—carbon atom compounds of the tri carboxylic acid cycle

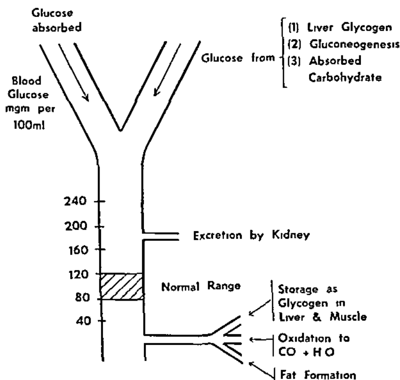


Fig 47 Factors regulating the level of blood glucose

CHAPTER X

THE INTERNAL SECRETIONS OF THE PANCREAS

CARBOHYDRATE METABOLISM

Introduction

The term carbohydrate metabolism may be taken to include all the biochemical reactions to which the carbohydrates of the body are subjected together with certain chemical changes which result in the production of carbohydrate from protein and fat The carbohydrates of the body include those ingested in the food and those formed in the body from noncarbohydrate sources i e

1 *MONOSACCHARIDES* (a) Hexose sugars (i) Glucose
(ii) Fructose
(iii) Galactose
(b) Pentose sugars — Ribose and others

2 *DISACCHARIDES* (a) Maltose
(b) Sucrose
(c) Lactose

3 *POLYSACCHARIDES* (a) Starch
(b) Glycogen

However, the metabolism of the disaccharides and polysaccharides other than glycogen and lactose, is completed before they leave the alimentary canal where they are hydrolysed to glucose fructose and galactose The subsequent metabolic processes are concerned therefore primarily with monosaccharides with the compounds into which they may be converted and the compounds from which they may be formed These compounds include —

(i) Glycogen which is composed of an unknown number of glucose molecules

(ii) A number of compounds intermediate between glucose on the one hand and carbon dioxide and water on the other These include —

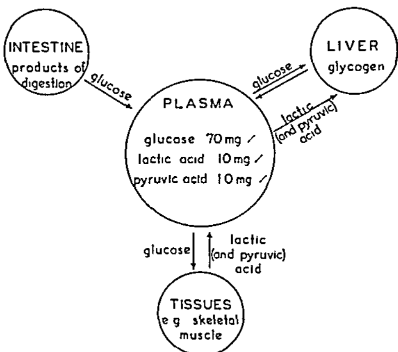


Fig 48 Diagram showing the interchanges of glucose lactic acid and pyruvic acid between plasma and the structures which take part in the metabolism of carbohydrate (*Fulton Textbook of Physiology*)

excreted into the glomerular filtrate at a rate which exceeds the capacity of the tubules to reabsorb it and so it appears in the urine Under these conditions the renal threshold for glucose is said to have been exceeded A number of factors influence the level of the renal threshold For example it varies from one individual to another prolonged hyperglycaemia causes a rise in the renal threshold for glucose and thyroid hormone also appears capable of elevating the level of this threshold Both the rate at which glucose is added to the plasma and the rate of its removal are regulated in large measure by the hormones of certain endocrine glands including the adenohypophysis the islets of Langerhans the adrenal medulla and the adrenal cortex

Fate of Glucose

The glucose of blood can be dealt with in four possible ways

(1) Conversion to liver glycogen

(iv) *Neutral fats formed from carbohydrates*

(v) Glycerol

(vi) Amino acids from which glucose may be produced

Distribution of Carbohydrate Within the Body

Glucose and glycogen are the principal carbohydrate compounds in the body Glucose is the important carbohydrate of the blood and extracellular fluid glycogen is stored chiefly in the liver and in muscle but is widely distributed throughout the body Glucose is an active compound used in the chemical reactions which make use of carbohydrate as a source of energy On the other hand glycogen which is the animal equivalent of starch is a stable compound suitable for the storage of carbohydrate (Fig 48)

Blood Sugar

Glucose is present in normal human blood in concentrations which vary between 60 mgm per 100 ml in a fasting state and 120 mgm per 100 ml after a meal rich in carbohydrates The level of blood glucose at any given time represents the outcome of a dynamic equilibrium between glucose entering the blood from the alimentary canal and the liver on the one hand and glucose leaving the blood to enter the liver brain and muscle on the other (Fig 47) When glucose enters or leaves the blood at such a rate as to threaten the stability of the concentration of glucose in the blood (i e its ability to remain between 60 and 120 mgm per 100 ml) mechanisms are brought into play to remove or add glucose from or to the blood respectively In either case it is the liver which provides this stabilising influence[1] The liver owes its importance in the control of the concentration of blood sugar to its ability to convert glucose into glycogen on the one hand and to convert glycogen and certain amino acids into glucose on the other

If the blood glucose falls below a level of about 40 mgm per 100 ml a series of characteristic changes result which are initiated by the effects of hypoglycaemia upon the cells of the central nervous system and by the release of adrenaline from the adrenal medulla These changes include weakness hunger sweating vaso constriction tremor convulsions and coma which will end in death unless this train of events be interrupted by the administration of carbohydrate

On the other hand a blood sugar level exceeding 120 mgm per 100 ml evokes no change of itself If the hyperglycaemia be sufficiently severe (usually over 180 mgm per 100 ml) glucose is

amount needed to meet the immediate requirements of the tissues This it does by means of a series of reactions which together constitute one important aspect of carbohydrate metabolism The reactions involved are as follows

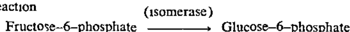
(i) Glucose + Adenosine triphosphate $\xrightarrow{\text{(hexokinase)}}$ Glucose-6-phosphate + Adenosine diphosphate

(ii) Glucose-6-phosphate $\xrightleftharpoons{\text{(phosphoglucomutase)}}$ Glucose-1-phosphate

(iii) Glucose-1-phosphate $\xrightleftharpoons{\text{(phosphorylase)}}$ Glycogen + PO_4

(iv) Adenosine diphosphate + PO_4 $\longrightarrow$ Adenosine triphosphate

Adenosine triphosphate acts as a phosphate donor but is reconstituted by reaction (iv) in which the phosphate is returned thereby preventing the accumulation of phosphate which brings this series of reactions to a standstill in a test tube reaction (iv) is also controlled by an enzyme system The presence of some glycogen is necessary to start this sequence of chemical reactions Fructose can enter the same series of reactions because the liver possesses the enzyme isomerase which stimulates the following reaction



Fructose-6-phosphate $\xrightarrow{\text{(isomerase)}}$ Glucose-6-phosphate

Release of Glucose

On the other hand when the tissues require glucose the liver is called upon to make good this need The liver accomplishes this by two means (a) it reverses the reactions by which glucose is converted to glycogen and so liberates glucose into the blood (glycogenolysis) and (b) it produces glucose from noncarbohydrate sources (gluconeogenesis)

(a) The release of glucose from glycogen consists essentially of the reversal of the reactions by which the liver forms glycogen from glucose

(i) Glycogen + PO $\xrightleftharpoons{\text{(phosphorylase)}}$ Glucose-1-phosphate

(ii) Glucose-1-phosphate $\xrightleftharpoons{\text{(phosphoglucomutase)}}$ Glucose-6-phosphate

(iii) Glucose-6-phosphate $\xrightarrow{\text{(phosphatase)}}$ Glucose + PO_4

(ii) Conversion to glycogen in other tissues (notably muscle)
(iii) Conversion to water and carbon dioxide with the production of heat and energy
(iv) Conversion to fatty acids (Figs 47 and 49)

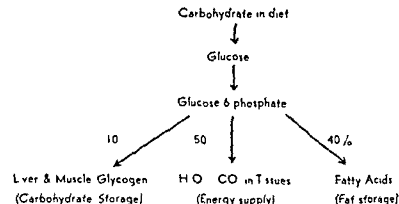


Fig 49 The fate of glucose during the ingestion of a diet rich in carbohydrate

The following terms are frequently used in describing the metabolic pathways of carbohydrate

(i) *GLYCOGENOLYSIS* This may be defined as the destruction of glycogen within the body It consists essentially of the conversion of glycogen to glucose

(ii) *GLUCONEOGENESIS* refers to those metabolic processes by which glucose is formed from noncarbohydrate sources (e g from amino acids and glycerol)

(iii) *GLYCOLYSIS* is a word used to denote the anaerobic conversion of glycogen to lactic acid It may be defined as the sum total of the reversible enzymatically controlled reactions which break down glucose to pyruvic acid and lactic acid

(iv) *GLYCOGENESIS* may be defined as the formation of glycogen from glucose

(v) *GLUCOGENESIS* is the formation of glucose from glycogen and is essentially the same as glycogenolysis since under aerobic conditions glycogen is converted to glucose (Fig 48)

Conversion of Glucose to Glycogen

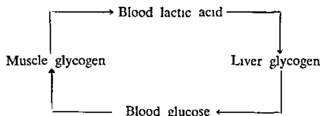
In order to store ingested glucose for future needs the liver converts to glycogen that present in the blood in excess of the

SKELETAL MUSCLE Muscle can synthesise glycogen from glucose and can store the glycogen against times when blood glucose is inadequate for the needs of the moment The reactions by which muscle converts glucose to glycogen are essentially the same as those seen in the case of the liver (page 201) The breakdown or hydrolysis of muscle glycogen begins with similar reactions to those seen in the liver Two general pathways are available to the muscle for the hydrolysis of glycogen —

(i) *Aerobic* Aerobic metabolism is qualitatively the more important and derives from the carbohydrate molecule all of its potential energy

(ii) *Anaerobic* More is known about anaerobic metabolism of glycogen this yields two molecules of lactic acid which represent an energy content of about nine tenths of the original glucose It is therefore important to the economy of body energy that lactic acid be not wasted

There are two pathways by means of which lactate may be reclaimed In the presence of oxygen lactate may be oxidised to pyruvic acid after which it can re-enter the usual catabolic channels or may be resynthesised to glycogen Secondly lactate which is highly diffusible may leave the muscle and diffuse into the blood in which it is conveyed to the liver In the liver lactate is converted to glycogen and subsequently to glucose This second method of lactate disposal is referred to as the Cori cycle

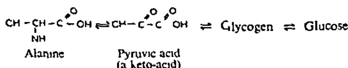


Lactic and pyruvic acids are diffusible and unlike other intermediary products of muscle metabolism they leave the muscle and enter the blood they do this in greater concentrations during anaerobic utilization of carbohydrates as in strenuous exercise or at high altitudes The extent of the rise in blood levels of lactate and pyruvate is a measure of the extent of anaerobic glycolysis Following exercise the production of both lactate and pyruvate falls and the liver gradually removes excess of both from the blood until blood levels return to normal

The glucose so formed diffuses out of the liver cell into the blood within the liver sinusoids provided the concentration of glucose in that blood be lower than in the intracellular fluid of the liver cells

(b) The liver can convert certain amino acids and the glycerol portion of fat molecules into glucose The term gluconeogenesis indicates the noncarbohydrate origin of this glucose The glucose derived from such sources is indistinguishable from that obtained from glycogen and it shares the same metabolic fate Amino acids are more important than glycerol as a source of glucose there is no evidence that under normal conditions fat constitutes a significant source of glucose Some amino acid is normally being converted to carbohydrate just as some carbohydrate is normally being used in amino acid synthesis However gluconeogenesis is especially important during fasting or during periods of low carbohydrate intake The cells of certain tissues (including the central nervous system) are so dependent upon glucose as a source of energy that they could not survive even a brief period of fasting were it not possible for the liver to convert amino acids to glucose The chemistry of this conversion is not fully understood but is thought to involve oxidation with the formation of a keto acid

(Keto C = O Acid C(=O)–OH) e g —



Peripheral Utilization of Carbohydrate

Glucose is distributed to the cells of the body where it may be *used to provide* heat and energy or it may be stored according to the nature of the tissue and the needs of the moment These two possibilities embrace the second and third means of dealing with glucose (page 200) The details of this phase of carbohydrate metabolism are incompletely understood but something is known of the way in which muscle deals with glucose and this tissue serves as an illustration of the peripheral utilization of carbohydrate although no doubt differences exist between one tissue and another Some of these differences are understood in a general way but the details of carbohydrate metabolism in tissues other than muscle await further study

This cycle may be illustrated as follows

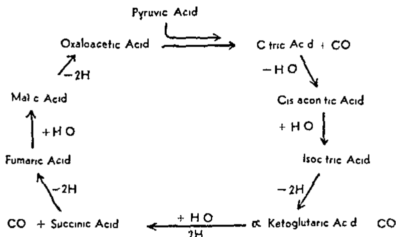
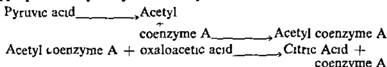


Fig 50 The Krebs or citric acid cycle

Pyruvic acid actually enters the cycle by formation of acetyl coenzyme A which in the presence of oxaloacetic acid and an appropriate enzyme yields coenzyme A and citric acid



From here the Krebs cycle proceeds as shown (Fig 50) It should be pointed out that the three major types of food stuff can give rise to the 2 carbon atom fragments called acetyl and from here they enter a final common metabolic pathway In addition acetyl coenzyme A may give rise to acetoacetic acid or to the formation of fatty acids and cholesterol (Fig 51) Thus the metabolism of carbohydrate is linked with that of protein and fat in such a way that the body is able to make use of ingested fuel whether this be predominantly fat carbohydrate or protein Without this capacity for the ready interconversion of the major food stuffs body metabolism would be severely compromised unless the food ingested were calculated to meet the needs of the moment

Nervous Control of Carbohydrate Metabolism

It has been known for over one hundred years that experimental lesions in the region of the hypothalamus may cause hypergly-

Much less is known about the details of the reactions by which tissues other than muscle make use of carbohydrate Throughout this chapter the word tissues is used where it would be more accurate to speak of muscle because other tissues may not necessarily behave towards carbohydrates in the same way

HEART MUSCLE Heart muscle shows the same metabolic pathways for the production of energy from carbohydrates as those described for skeletal muscle It was once thought that the break down of glycogen to lactic acid did not occur in heart muscle except under conditions of extreme anoxia Much doubt has been cast upon this view by recent work and it may be that lactic acid is the most important fuel of the heart under normal conditions Unlike skeletal muscle the heart can contract only a very small oxygen debt

CENTRAL NERVOUS SYSTEM The cells of the central nervous system depend almost entirely upon carbohydrate as an immediate source of energy Most other tissues can derive a portion of their major needs from fat and amino acids However the rate of carbohydrate utilization by the nervous system is independent of insulin with the result that although this tissue is completely dependent upon glucose for its metabolic needs it achieves some degree of autonomy and is not entirely at the mercy of extracranial tissues

The Final Common Pathway of Metabolism

Pyruvic acid may arise from the catabolism of carbohydrate protein and fat It is therefore a key substance in the interconversion of protein carbohydrate and fat as well as in the chemical reactions by means of which the body makes use of the energy of carbohydrate Pyruvic acid gives rise to acetyl by decarboxylation (loss of CO) and union of acetyl with coenzyme A then takes place producing acetyl coenzyme A this is an irreversible oxidation and occurs in liver and muscle Acetyl is the name given to the 2 carbon atom fragments split off from various molecules during their metabolism in the body Pyruvic acid and fatty acids are among the important sources of acetyl Acetyl coenzyme A may then enter a series of reactions known either as the tricarboxylic acid cycle of Krebs or the citric acid cycle in which oxidation progresses through a series of deliberate steps from which energy is released and utilized

Embryology

During the fifth week of embryonic life two outpocketings of the duodenum appear One is called the dorsal pancreas and grows rapidly During the seventh week it is joined by the smaller ventral pancreas which is destined to form the head of the adult pancreas these two structures fuse

During the fourth month budding of the ducts causes acini to develop and at the same time by the same process of budding islets begin to develop At first the islets are single sprouts but later they grow into complex masses About one million islets form in man and some retain their impervious connection with the ducts

Histology

The islets are irregular structures with an extensive blood supply and are delimited by a thin reticular membrane They are composed of cords of irregularly prismatic cells which are conspicuously pale beside the cells of the acini The most prominent and numerous cells are called beta cells these contain alcohol soluble granules Alpha cells are arranged near the periphery of the islets and contain acidophil granules The gamma cells are nongranular while the delta cells are scanty and have been found only in man

Function

The function of the islets of Langerhans is to secrete one or perhaps two hormones Beta cells produce insulin and it is likely that alpha cells produce glucagon

Insulin

CHEMISTRY Insulin is a protein and the order of its constituent amino acids has now been determined (page 23) It has been obtained in crystalline form containing small amounts of zinc Insulin does not appear to have a prosthetic group but depends for its activity upon the structure of the molecule as a whole[1]

METABOLISM Insulin is stored in the granules of the β cells and released into the circulation as required In plasma a proportion of the insulin is bound to protein the protein bound form is in equilibrium with free insulin and as the latter is used by the tissues it is replaced from the bound form

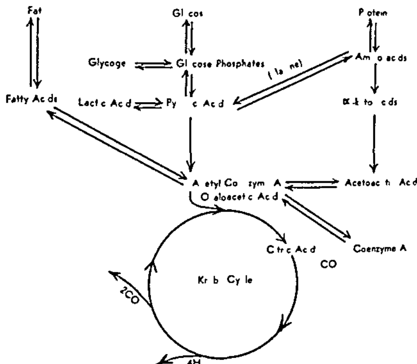


Fig 51 Diagram to illustrate interconversions among the three basic food stuffs Most of these interconversions occur within the liver but the oxidation reactions take place in all tissues (*Fulton*)

caemia The mechanism of this response is uncertain but it has been suggested that such lesions cause irritation of autonomic nerve centres within the hypothalamus which in turn stimulate the adrenal medulla to secrete adrenaline It appears likely that irritation rather than destruction of nerve centres is responsible for the hyperglycaemia since brain sections above the pons do not produce this response Beyond the fact that the central nervous system exerts some influence upon carbohydrate metabolism nothing is known about the nature and extent of this influence

PANCREAS

The pancreas is a double structure in man in that the bulk of the gland produces an external secretion concerned with digestion while certain scattered groups of cells called the islets of Langerhans, produce at least one internal secretion

action of hexokinase It must be said however that final proof is lacking for this view of the site of insulin activity and other suggestions have received some experimental support Nevertheless it is convenient at this stage of our knowledge to regard insulin as stimulating the action of hexokinase

Recent experimental evidence suggests that insulin accelerates the passage of glucose across cell membranes This passage may involve a reversible reaction as the result of which glucose forms a complex with some carrier molecule at the outer surface of the cell free glucose is released at the inner surface of the cell membrane The relative importance of these two actions of insulin remains to be determined

It can therefore be seen that carbohydrate metabolism does not come to a standstill in the absence of insulin because like other hormones it merely regulates the rate of chemical reactions which can proceed (although much more slowly) in its absence This is not to suggest that insulin is unimportant in body economy Diabetes mellitus is a disease which results from failure of insulin secretion and under these conditions gross changes in body metabolism are observed

BODY METABOLISM IN THE ABSENCE OF INSULIN In the absence of the regulatory action of insulin the following metabolic changes occur —

(i) *Hyperglycaemia* Raised blood sugar is more evident when a normal diet is taken than during periods of fasting but in any case the blood sugar eventually rises in the absence of insulin

(ii) *Glycosuria* After a time the hyperglycaemia exceeds the renal threshold and glucose appears in the urine

(iii) *Diuresis* The excretion of glucose carries with it a large volume of water this is an osmotic phenomenon

(iv) *Disposal of blood glucose* In the absence of insulin, changes occur in the disposal of blood sugar

- (a) Conversion to glycogen appears to be defective in the absence of insulin however the importance of this effect has been challenged in recent years[15]
- (b) The rate of oxidation of glucose by the tissues diminishes [7]
- (c) The conversion of carbohydrate to fat becomes very slow[8 9 10]

(v) *Ketogenesis* As a result of retardation in the catabolism of carbohydrate the organism is forced to derive most of its

Insulin is inactivated in the tissues (especially in the kidney and liver) by an enzyme called insulinase There is also some evidence for the existence of an inhibitor of insulinase

ACTION Insulin is not known to have any function other than the part it plays in carbohydrate metabolism here it promotes the removal of glucose from the blood and the following effects are observed after injections of insulin —

1 An increase in the conversion of glucose to glycogen in the liver[4,5]
2 An increase in the conversion of glucose to glycogen in the muscle[4,5]
3 An increase in the oxidation of glucose by the tissues[6,7]
4 An increase in the rate of conversion of carbohydrate to fat[8,9,10,11]
5 An increase in the rate of protein synthesis

In other words directly or indirectly insulin stimulates the removal of glucose from the blood by the four available routes of disposal (page 199) Under normal conditions the fourth effect is probably the most important quantitatively It seems probable that the fundamental action of insulin is to promote the conversion of glucose to glucose 6 phosphate a reaction which is catalysed by the enzyme hexokinase In this way insulin speeds up the rate of conversion of glucose to glycogen and also the rate at which the tissues burn sugar The conversion of glucose to glucose 6 phosphate is essentially irreversible during the conversion of glucose to glycogen and it is therefore a logical place for a hormone to regulate the rate of reaction[1,13,14] Of course glucose 6 -phosphate can be converted to glucose under the influence of *another enzyme (page 201) but this conversion takes place* in a different context Generally speaking enzymes stimulate reactions in two directions and the direction elected by a given reaction at a given time is the outcome of the relative concentrations of the reacting substances together with the rate of their removal from the site of the reaction

However when a biochemical reaction involves a great change in potential chemical energy, it is usually not reversible and enzymes promote only the down hill reaction that is towards a decrease in free energy Among the reactions which convert glucose to glycogen the first step (the conversion of glucose to glucose 6 phosphate) is controlled by the enzyme hexokinase in *this one direction only because of the high energy level* of adenosine triphosphate Insulin is thought to promote this

to glucose in the liver It seems probable that this action results from the activation of liver phosphorylase

In addition to its action in the liver glucagon has been shown to inhibit gastric contractions and to relieve hunger The hormone also enhances the renal clearance of sodium chloride potassium and phosphate Moreover glucagon increases the peripheral utilization of glucose and for this reason should not be referred to as an antagonist of insulin It has also been shown that the combined use of insulin and glucagon causes a greater increase in the peripheral utilization of glucose than the use of either alone These observations suggest that the two hormones can together stimulate the utilization of glucose and at the same time maintain the concentration of glucose in blood with greater constancy than would be possible in the absence of either hormone The secretion of glucagon appears to be controlled by the concentration of glucose in the blood and by the level of blood insulin A rise in blood insulin and a fall in blood glucose stimulates the secretion and the release of glucagon It is also likely that glucagon may regulate its own secretion by direct action upon the alpha cells Although there is some evidence to show that growth hormone stimulates the secretory activity of the alpha cells this evidence is not conclusive

THE ROLE OF THE ENDOCRINE GLANDS IN CARBOHYDRATE METABOLISM

Apart from the pancreas the adenohypophysis the adrenal medulla the adrenal cortex and the thyroid gland exert important effects upon carbohydrate metabolism

ADENOHYPOPHYSIS The two hormones of the adenohypophysis which are important in carbohydrate metabolism are growth hormone and ACTH Certain effects have been described following the injection of extracts of the anterior pituitary gland e g pancreatotrophic and diabetogenic actions It has been pointed out already (page 142) that these actions do not necessarily prove the existence of specific hormones TSH may influence carbohydrate metabolism indirectly

1 *Growth Hormone* Growth hormone stimulates growth in young animals but in some species it produces hyperglycaemia after maturity (page 145) This hyperglycaemia is associated with other changes characteristic of diabetes and is brought about as the result of the following actions [7] (Fig 52)

energy by burning fat This results in an increase in the production of ketone bodies (acetoacetic acid acetone hydroxy butyric acid) The rate of production of ketone bodies comes to exceed the rate at which they can be used by the body so that they accumulate in the blood and are excreted in the urine[13 14] Ketone bodies are neutralised by the buffers of the blood and are excreted as sodium and potassium salts This leads to a considerable loss of base from the body and eventually to acidosis

(vi) *A negative nitrogen balance occurs* this results from the destruction of body protein The loss of protein is an indirect result of diminished use of carbohydrate

CONTROL OF INSULIN SECRETION

(i) The chief controlling influence over the production of insulin is the concentration of glucose in the blood[16] An elevation of the concentration of glucose in the blood of the pancreatic arteries leads to an increased secretion of insulin Cross circulation experiments suggest that this is the result of a direct effect of blood glucose upon the islet cells The reverse namely a fall in insulin secretion in response to low blood sugar may occur but is more difficult to establish

(ii) The islets are supplied by the vagus nerve and it has been suggested that the hypothalamus may affect the secretion of insulin by means of this nerve Such an effect if it does exist appears to be of secondary importance in normal individuals

(iii) Humoral factors appear to be important in the control of insulin Insulin suppresses its own secretion while glucagon increases the production of insulin Growth hormone appears to stimulate insulin production when first injected into animals but repeated doses depress the secretion of insulin There is some evidence to show that androgens facilitate insulin secretion while oestrogens have the opposite effect

Glucagon

Glucagon is the name given to a glycogenolytic hormone secreted by the α cells of the pancreatic islets Glucagon is a protein consisting of a straight chain of 29 amino acids with histidine as the N terminal acid and threonine as the C terminal acid The principal action of glucagon is to stimulate the conversion of glycogen

normal (page 192) This is achieved by the following actions (*Fig* 53)

(i) Adrenaline stimulates the conversion of glycogen to glucose in the liver

(ii) It increases the rate of oxidation of glycogen to lactic acid in the tissues This lactic acid is conveyed to the liver where it is used to replenish stores of glycogen and so indirectly assists in raising the level of blood sugar

(iii) Adrenaline stimulates the release of ACTH from the adenohypophysis and thereby causes an increase in the production of glucocorticoids by the adrenal cortex (Fig 38)

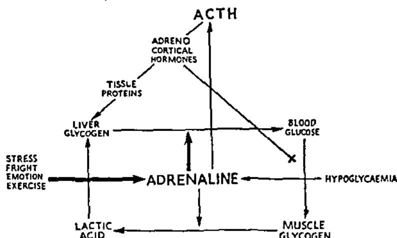


Fig 53 The action of adrenaline on carbohydrate metabolism (*P M F Bishop*)

ADRENAL CORTEX The role of the adrenal cortex in carbohydrate metabolism is due to the action of glucocorticoids which as their name implies have important effects upon this metabolism [8] (Fig 52)

(i) Glucocorticoids depress the rate of reabsorption of glucose from the renal tubules and thereby promote glycosuria

(ii) They bring about an increase in the destruction of protein and in the conversion of amino acids so liberated into carbohydrate Some workers believe that glucocorticoids do not so much destroy protein as interfere with its synthesis from amino acids the excess of amino acids created by this block in protein synthesis is converted to glucose In either event the hormones promote gluconeogenesis (Fig 52)

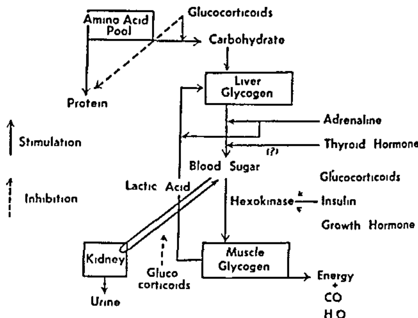


Fig 52 Diagram to illustrate the effects of hormones on carbohydrate metabolism

(1) Growth hormone opposes the action of insulin in stimulating hexokinase activity This interferes with the capacity of the liver to convert glucose to glycogen In other words one very important means of the disposal of the glucose present in the blood beyond *the needs of the moment is removed This leads to hypergly* caemia and to the appearance of glucose in the urine when the renal threshold is exceeded

(2) At first growth hormone stimulates the beta cells of the pancreas to produce more insulin but not in sufficient quantity to cope with the demand produced by the first action

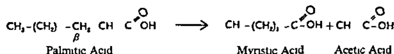
(3) Later the beta cells undergo exhaustion atrophy and per manent diabetes sets in

(4) The alpha cells continue to produce a hyperglycaemic factor (glucagon) since they are unaffected by growth hormone

2 *ACTH* The part played by ACTH in carbohydrate metabolism *results from its capacity to stimulate the production of* glucocorticoids by the adrenal cortex

ADRENAL MEDULLA The secretion of adrenaline by the medulla is stimulated by hypoglycaemia and constitutes part of an emergency mechanism to bring the blood glucose level back to

When large quantities of fat are burnt by the body in the absence of adequate metabolism of carbohydrate a pathway of oxidation is used which leads to the accumulation of intermediary compounds known as ketone bodies (β hydroxybutyric acid acetoacetic acid and acetone) These may reach such concentrations in the body as to interfere with acid base balance leading eventually to acidosis Under these circumstances the breakdown of fatty acids proceeds by oxidation of the β carbon atom (i e that next but one to the acid groups $C{\overset{O}{\leftarrow}}OH$ In this way the last two carbon atoms of the molecule are split off as acetic acid and a new fatty acid is formed with two carbon atoms less than its predecessor, e g



The naturally occurring fatty acids contain an even number of carbon atoms so that they are ultimately broken down to butyric acid ($CH_3-\underset{\beta}{CH}-\underset{\alpha}{CH}-C\overset{O}{-}OH$) which can be converted either to β hydroxybutyric acid ($CH_3\;\underset{OH}{CH}\;CH\;C\overset{O}{}OH$) or to acetoacetic acid ($CH_3-C\overset{O}{}CH-C\overset{O}{-}OH$) These two compounds are inter-convertible in the body and acetoacetic acid is highly toxic The third ketone body acetone ($CH-\underset{O}{\overset{\|}{C}}\;CH$) is formed from acetoacetic acid Ketone bodies are produced in the liver and can be used by the tissues as a source of energy This method of oxidation of fatty acids occurs in normal subjects but because of the inability of the diabetic to use adequate quantities of carbohydrate β-oxidation is carried to such extremes that acetone bodies accumulate in the blood and exert their toxic effects in addition to causing acidosis

Although diabetes may occur as a complication of diseases of the adrenal cortex or of the adenohypophysis the cause of the condition in most cases remains uncertain Apart from the possibility of inadequate secretion of insulin excessive inactivation by insulinase and abnormal binding in the plasma have been considered

Within recent years the search for drugs which can be used in

(iii) Glucocorticoids *appear to inhibit the stimulating effect of* insulin upon the activity of hexokinase In this way they interfere with the utilization of glucose by the tissues

THYROID GLAND Thyroid hormone plays some part in the control of carbohydrate metabolism It is known to exert the following effects (see page 63)

(i) Thyroid hormone accelerates the rate of absorption of carbohydrate from the alimentary tract Excess thyroid hormone in the blood produces an abrupt, temporary rise in blood sugar after the ingestion of carbohydrate This rise is followed by a rapid return to normal

(ii) Thyroid hormone stimulates the metabolic activity of all cells and no doubt this action involves an acceleration of carbohydrate metabolism The point at which this accelerating factor operates is uncertain

(iii) Excess of the hormone also *depletes the liver stores* of glycogen This may result from increased oxidation of glucose by the cells of the body Furthermore, thyroid hormone prevents the deposition of glycogen in the liver during the process of refeeding after starvation In short the fundamental action of thyroid hormone on carbohydrate metabolism is to promote the mobilization of liver glycogen

In addition the hormone appears to accelerate the activity of *the cells of the renal tubules and thereby promotes* the *reabsorption* of glucose i e it raises the renal threshold for glucose The observations upon which these statements are based concern the state of affairs prevailing in the presence of abnormal blood levels of thyroid hormone The importance of this hormone in the regulation of carbohydrate metabolism under physiological conditions cannot be stated

DIABETES MELLITUS

When the pancreas of an animal is completely removed a series of characteristic symptoms develop which eventually lead to death The same symptoms appear spontaneously in patients afflicted with the disease diabetes mellitus which involves inadequate production of insulin by the pancreas The concentration of glucose in the blood begins to rise and eventually exceeds the renal threshold with the result that glycosuria follows The body fails to make sufficient use of glucose and therefore calls upon fat and protein to provide the energy required for day to day living

(ii) Cerebral cortical involvement is indicated by paraesthesiae and numbness of the extremities together with twitching double vision aphasia amnesia apraxia involuntary micturition and convulsions

(iii) Thalamic symptoms include compulsion to laugh or cry speaking at the top of the voice choreiform movements and grimaces The subject knows he should take sugar but is unable to put anything in his mouth or to reach for food in front of him

The clinical picture of hypoglycaemia is extremely variable and it is not surprising that the condition is sometimes mistaken for alcoholic intoxication

TESTS OF CARBOHYDRATE METABOLISM

GLUCOSE TOLERANCE TEST When glucose is taken by mouth it passes quickly into the small intestine where it is absorbed The absorption of glucose is associated with a rise in the level of blood sugar which gradually returns to its previous concentration within two hours The glucose tolerance test involves the administration of 100 grams of glucose to a fasting subject Blood sugar levels are estimated in samples taken immediately before the glucose is given and at intervals of one half hour one hour two hours and three hours after the ingestion of the glucose The figures obtained are plotted against time (Fig 54) and the resulting curve depends upon

1) The rapidity of absorption of glucose from the alimentary canal
2) The capacity of the body to store glucose and of the tissues to utilize sugar
3) The rate of discharge of glucose from the liver

The urine is examined for the presence of reducing substances at the time each blood sample is taken

In the disease diabetes mellitus the removal of the test dose of sugar from the blood stream is slow The resulting curve rises to high levels and returns to fasting levels more slowly than normal (Fig 54) Rapid absorption of glucose from the alimentary canal causes a sharp rise and fall in blood glucose levels this is seen in hyperthyroidism and after gastrectomy In the presence of an excessive secretion of insulin the fall of blood sugar concentrations is excessively rapid and the level reached is below 80 mgm per 100 ml

the treatment of diabetes and are active when taken by mouth has in some measure been rewarded The first of these antidiabetic substances to be used was derived from the sulphonamide drugs The mechanism by which these sulphonamide derivatives exert their antidiabetic properties is uncertain but three possible explanations have been supported by experimental data (i) they may depress the secretion of glucagon (ii) they may diminish the activity of insulinase in the liver or (iii) they may enhance the secretion of insulin

HYPOGLYCAEMIA

While excessive muscular exertion after a period of fasting may produce low blood sugar the same result may follow overactivity of the islets of the pancreas which secrete an excess of insulin in response to the ingestion of carbohydrate Again diffuse disease of the liver may interfere with glycogenolysis and so produce hypoglycaemia a condition which may also be produced by injecting insulin into normal subjects Hypoglycaemia produces an increase in the rate of secretion of adrenaline and affects the function of the central nervous system

The symptoms of hypoglycaemia are characteristically ushered in by hyperexcitability a desire for food and a sense of impending danger There follow a series of symptoms the relative severity of which varies from one person to another and eventually lead to coma *The intervening symptoms may be classified as follows*

(A) *SYMPTOMS DUE TO THE SECRETION OF ADRENALINE*

(i) Sweating is usually noticeable and may lead to drenching of the clothes while tremor is common *

(ii) Rapid pulse and a rise in blood pressure are usual

(iii) *Vomiting and lower abdominal pain may be due to splanch*nic vasoconstriction

(iv) A sense of apprehension is attributable to the action of adrenaline upon the central nervous system

(B) *SYMPTOMS DUE TO DISTURBANCE OF CENTRAL NERVOUS FUNCTION*

(i) *Disturbances of the function of the higher centres produce* excitement anxiety depression and sometimes attacks of violence

*Adrenaline does not act directly upon the sweat glands and the mechanism of hypoglycaemic sweating remains obscure

(ii) Cerebral cortical involvement is indicated by paraesthesiae and numbness of the extremities together with twitching double vision aphasia amnesia apraxia involuntary micturition and convulsions

(iii) Thalamic symptoms include compulsion to laugh or cry speaking at the top of the voice choreiform movements and grimaces The subject knows he should take sugar but is unable to put anything in his mouth or to reach for food in front of him

The clinical picture of hypoglycaemia is extremely variable and it is not surprising that the condition is sometimes mistaken for alcoholic intoxication

TESTS OF CARBOHYDRATE METABOLISM

GLUCOSE TOLERANCE TEST When glucose is taken by mouth it passes quickly into the small intestine where it is absorbed The absorption of glucose is associated with a rise in the level of blood sugar which gradually returns to its previous concentration within two hours The glucose tolerance test involves the administration of 100 grams of glucose to a fasting subject Blood sugar levels are estimated in samples taken immediately before the glucose is given and at intervals of one half hour one hour two hours and three hours after the ingestion of the glucose The figures obtained are plotted against time (Fig 54) and the resulting curve depends upon

1) The rapidity of absorption of glucose from the alimentary canal
2) The capacity of the body to store glucose and of the tissues to utilize sugar
3) The rate of discharge of glucose from the liver

The urine is examined for the presence of reducing substances at the time each blood sample is taken

In the disease diabetes mellitus the removal of the test dose of sugar from the blood stream is slow The resulting curve rises to high levels and returns to fasting levels more slowly than normal (Fig 54) Rapid absorption of glucose from the alimentary canal causes a sharp rise and fall in blood glucose levels this is seen in hyperthyroidism and after gastrectomy In the presence of an excessive secretion of insulin the fall of blood sugar concentrations is excessively rapid and the level reached is below 80 mgm per 100 ml

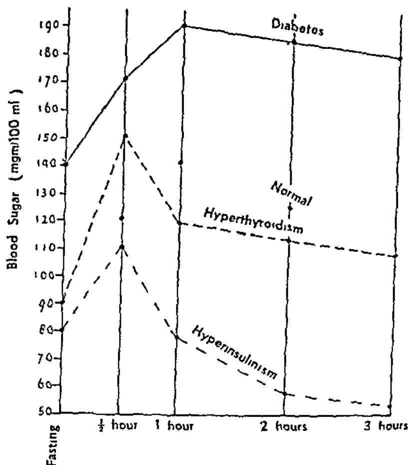


Fig 54 Graph illustrating the glucose tolerance test under various conditions

Experimental Procedures in the Study of Carbohydrate Metabolism

In the experimental studies of normal and abnormal carbohydrate metabolism two substances have proved to be of great practical value namely alloxan and phloridzin

ALLOXAN is a synthetic derivative of uric acid which produces destruction of the beta cells of the pancreas By means of alloxan injections a *form of experimental diabetes can be produced* without any other side effects such as those which follow removal of the pancreas and without the necessity of an abdominal operation Alloxan diabetes appears to resemble the disease diabetes mellitus

very closely and animals rendered diabetic in this way have been of great value in the experimental study of this disease

PHLORIDZIN is a substance which causes a prompt temporary lowering of the renal threshold for glucose In this way the compound produces glycosuria without so far as can be seen producing any other change in the body Like alloxan phloridzin has been of great use in experimental studies of carbohydrate metabolism

REFERENCES

1 Sanger F and Thompson E O P Biochem J 53 353 366 1953
2 Sutherland E.W and Cori C F J Biol Chem 188 531 1951
3 Mann F C., Medicine Baltimore 6 419 1927
4 Bridge E.M Johns Hopk. Hosp Bull 62 408 1938
5 Stadie W C. and Zapp J A. J Biol Chem 170 55 1947
6 Feeler D D et al. J Biol Chem 188 865 1951
7 Wick A N et al J Biol Chem 188 241 1951
8 Stetten D W Jr and Boxer G E J Biol Chem 156 271 1944
9 Stetten D W Jr and Klein B V J Biol Chem 159 593 1945
10 Stetten D W Jr et al. J Biol Chem 192 817 1951
11 Swensson A. Acta Physiol Scand 11 (Suppl 33) 1 1945
12 Colowick, S P., et al J Biol. Chem 168 583 1947
13 Stadie W C Physiol Rev 34 52 1954
14 Stadie W C et al J Biol Chem 184 617 1950
15 Bondy R O et al J Clin Invest 28 1216 1949
16 Foa, P P et al Amer J Physiol 157 197 1949
17 Young, F G Biochem J 39 515 1945
18 Long C N H Endocrinology 26 309 1940
19 Bishop P M F Recent Advances in Endocrinology p 8 Churchill London 1954
20 Foa P P Ciba Colloquia on Endocrinology Volume 9 1956

CHAPTER XI

THE PARATHYROID GLAND

Introduction

The parathyroid glands are usually four in number and each is about the size of a small pea However they are variable in number and in their position so that parathyroid tissue may be found almost anywhere between the base of the skull and the arch of the aorta The glands are intimately concerned with the metabolism of calcium and phosphorus

CALCIUM METABOLISM

1) *DISTRIBUTION* More than 99 per cent of the calcium stored in the body is to be found in the skeleton The remainder occurs in the extracellular fluid where it plays a vital role in a number of important physiological activities including

(i) the normal function of the voluntary and autonomic nervous system
(ii) coagulation of blood
(iii) maintenance of normal membrane permeability,
(iv) normal contraction of cardiac muscle
(v) neuromuscular excitability
(vi) bone metabolism

2) *ABSORPTION* Calcium is absorbed in the upper part of the small intestine Absorption is facilitated by the presence of hydrochloric acid and vitamin D it is hindered by the presence of alkali an excess of fat and phosphates and by the presence of phytic acid which is found in oatmeal and other cereals Fats and phosphates combine with calcium to form insoluble compounds which are less readily absorbed than other forms of calcium However in recent years it has been shown that very little calcium is absorbed no matter in what form it is ingested in the absence of vitamin D On the other hand vitamin D will very largely overcome those factors which hinder calcium absorption (i e fat phosphate and phytate) this is so whether the vitamin D be taken by mouth or given by intramuscular injection In short it would seem that in the final analysis the capacity of the body to

absorb calcium is largely a question of a balance between the amount of ingested vitamin D and the quantity of inhibitor substances in the diet

3) *FATE* Once calcium enters the circulation it is stored or excreted storage being chiefly in bone Calcium is excreted in the urine and in faeces It has generally been said that most of the calcium in faeces represents the unabsorbed residue of that which has been ingested but recent observations suggest that intestinal excretion of calcium may be quite considerable and that part of the faecal calcium may have been absorbed and re-excreted Certainly patients on diets which are virtually free of calcium continue to excrete considerable quantities of calcium in the faeces for some weeks The final answer to this question cannot yet be given A normal individual receiving adequate calcium in his diet excretes up to 20 per cent of ingested calcium in his urine Calcium is also lost through the placenta and the lactating breast The threshold

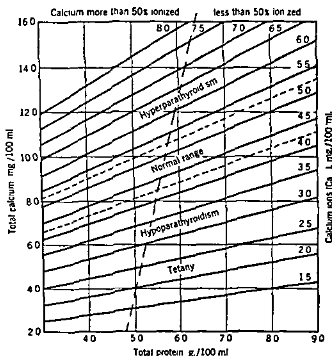


Fig 55 Chart for the determination of the concentration of calcium ions from the levels of total protein and total calcium of serum (*From McLean and Hastings (1935) with permission of the American Journal of Medical Science*)

for calcium excretion in the urine is thought to be about 7 mgm per 100 ml Adults receiving a low calcium diet usually excrete less than 150 mgm of calcium in their urine per day

4) *REQUIREMENTS* A normal adult requires about 0 5 gm of calcium daily a child and a pregnant woman each require about twice this amount while a lactating mother needs about 1 5 gm daily A normal diet without milk or cheese provides approximately 0 5 gm of calcium and one pint of milk contains about 0 65 gm of calcium

5) *CALCIUM IN THE BLOOD* The normal serum calcium value is 10 mgm per 100 ml plus or minus 1 mgm and this can be divided into three distinct fractions (Fig 55)

(i) *Protein bound calcium* This fraction is held in solution by the serum protein and is proportional in amount to the concentration of the serum protein At a serum protein concentration of 7 gm per 100 ml this protein bound fraction is about 4 mgm per 100 ml

(ii) A small portion of serum calcium is diffusible but not ionized This consists of a number of substances such as calcium citrate calcium salts of other organic acids and possibly colloidal tertiary calcium phosphate This fraction amounts to between 1 2 and 2 5 mgm per 100 ml

(iii) *Ionic calcium* The remaining part of the serum calcium (about 5 mgm per 100 ml) is present in the form of soluble calcium complexes or as a supersaturated solution of calcium phosphate and calcium carbonate This fraction is important for two reasons

1) It is physiologically active because it is ionized and can therefore diffuse into other extracellular fluids

2) It is under the control of the parathyroid glands

The calcium content of cerebrospinal fluid is approximately 5 mgm per 100 ml which represents the second and third fractions of serum calcium because the protein bound portion is not free to diffuse into such extracellular fluids In addition the amount of phosphate present in the serum influences the amount of calcium that can be held in solution Excess of phosphate in the serum causes a fall in the amount of calcium that can be held in solution and this excess precipitates out of solution A fall in serum phosphate level has the opposite effect

The estimation of ionic calcium in the serum is a tedious procedure but for most purposes it is sufficient to use the normo

gram shown in Fig 55 from which the level of ionic calcium can be read if the total serum calcium and total serum protein concentrations are known

PHOSPHORUS* METABOLISM

1) *DISTRIBUTION* About 80 per cent of the phosphorus of the body is stored in bone The remainder occurs either in the intracellular fluid of the protoplasm or in association with glycogen Phosphorus plays an important role in the following functions

(i) as a buffer in acid base balance
(ii) a constituent of coenzymes
(iii) carbohydrate metabolism makes use of compounds containing phosphorus
(iv) a constituent of such compounds as phospholipids nucleic acid nucleoprotein and phosphoproteins
(v) as an important constituent of bone

2) *ABSORPTION* Phosphorus is absorbed from the small intestine Absorption is facilitated by an excess of fat a diet low in calcium and by acids It is hindered by a high calcium diet and by alkaline salts the effect of a high calcium diet is overcome by the presence of sufficient vitamin D

3) *FATE* Once phosphorus enters the circulation it is either stored or excreted It may be stored in bone as a constituent of protoplasm or in the form of the compounds mentioned above Phosphorus is chiefly excreted in the urine but some faecal excretion occurs The faecal excretion of phosphorus is variable but is more important than was once thought As in the case of calcium the problem is to distinguish between unabsorbed phosphorus and excreted phosphorus and so far this has not been accomplished with certainty

4) *REQUIREMENTS* A normal adult requires 0 9 gm of phosphorus daily a child 1 5 gm and a lactating mother 2 5 gm daily

5) *PHOSPHORUS IN THE BLOOD* The serum phosphorus can be divided into 3 fractions

(i) Lipid phosphorus which constitutes 8 mgm per 100 ml
(ii) Ester phosphorus which amounts to 1 mgm to 100 ml
(iii) Inorganic phosphorus which represents about 3 mgm per 100 ml Therefore the total phosphorus content of serum is about 12 mgm per 100 ml The present discus

*Throughout this chapter the word phosphate refers to inorganic phosphorus

sion is limited to inorganic phosphorus, since only this fraction is completely ionized and is affected by the para thyroid glands The normal range of serum inorganic phos phorus is 2 7 to 3 7 mgm per 100 ml

Solubility Product

The product of the serum calcium and the serum inorganic phosphate levels in the serum is called the solubility product Within certain limits the serum levels of calcium and phosphorus exhibit a reciprocal relationship especially under the influence of parathyroid hormone The influence of this reciprocity upon the simultaneous deposition and resorption of bone may be illustrated by taking arbitrary figures When the solubility product is 50 an equilibrium exists and the resorption of bone exactly equals its deposition In the body fluid surrounding the surface of bone resorption the calcium may be 10 and the phosphate 3 and hence the solubility product is 30, this gives rise to a local under saturation which promotes resorption of bone Meanwhile, at areas of bone deposition alkaline phosphatase is produced by osteoblasts and this brings about a local increase in the concentration of phos phate which might rise to 6 giving a solubility product of 60 and hence a local condition of supersaturation this promotes the deposition of bone

The Composition of Bone

Bone consists of organic matrix and inorganic matter The structure of long bones has been closely studied but less is known of the composition of other types of bone When bone is allowed to stand in a solution of dilute acid the mineral portion dissolves leaving a strong flexible structure of organic material which retains the shape of the original bone The inorganic matter of bone imparts to this tissue an elastic modulus which has been com pared to that of concrete This mineral fraction of bone consists largely of calcium phosphate and carbonate 85 per cent of the calcium occurs in the form of tertiary calcium phosphate and 12 per cent as calcium carbonate The inorganic component of bone has been shown to exist in crystalline form which suggests that the salts concerned are deposited by precipitation (Fig 56) It has further been observed that regardless of variations in total mineral content of bone wherever bone is being laid down or resorbed the calcium phosphorus ratio remains practically fixed at about 2 2 to 1

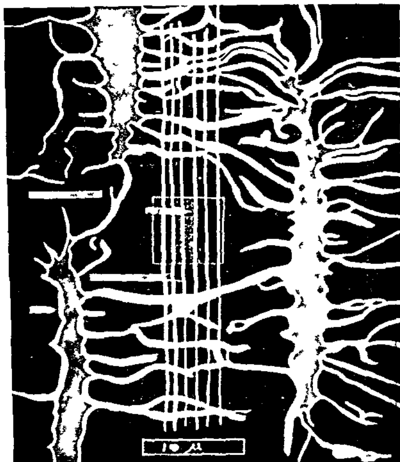


Fig 56 Diagrammati representation of the relationship between osteocytes collagen fibres cement substance and bone crystals The three osteocytes are seen set in a background of cement substance The inset shows the relationship between collagen fibres and bone crystals The syncytial network extending from the osteocytes enables the cement the collagen and the crystalline elements of bone to be brought under metabolic control The relationship shown between the collagen fibres and osteocytes is highly schematic in reality the fibres form a complex three dimensional network (*After R A Robinson J Bone and Joint Surg 34A 389 195*)

Bone salts The mineral fraction of bone consists chiefly of calcium phosphate in addition to calcium carbonate fluoride hydroxide and citrate Most of the magnesium and about one quarter of the sodium of the body are also present in bone Bone

crystals belong to the group of hydroxyapatites —



$$[Ca(OH)\ \ Ca_4(PO_4)_3]_2$$

Bone crystals are tabular, measuring approximately 500 by 250 by 100 A° The density of the microcrystal is about 3 0 and the surface area of 1 gram of the crystals is almost 100 square metres

Bone matrix Three-quarters of the volume and about half the weight of bone is made up by the organic matrix This matrix consists largely of a groundwork of osteocytes and collagen set in a gel of cement substance (Fig 56) Collagen is the fibrous protein of connective tissue and so far as can be determined the collagen fibres of bone do not differ from those of other fibrous tissue The cement of bone is similar to the ground substance of mesenchyme The elasticity which gives bone its capacity to respond to stress and strain is provided by collagen and elastic fibres together with the gel of the cement substance

Normal Bone Metabolism

The fundamental principle of bone metabolism is implicit in the observation that the adult skeleton is not the inert museum specimen of the anatomist but a living tissue which is constantly undergoing structural and metabolic change That is to say in some places bone is being formed and at the same time in other places it is undergoing resorption Both processes go on side by side and no matter whether the overall picture is one of formation or one of resorption both always occur together and the state of the skeleton at a given time is the outcome of the balance between deposition and resorption This state of affairs gives the skeleton a plastic quality which enables the individual bones to be moulded by the everyday stresses to which they are subjected The whole skeleton should be looked upon as a mass of protein impregnated with a crystalloid substance This mass is in a constant state of dynamic equilibrium with matrix formation and calcium salt deposition adding to it and matrix resorption together with calcium salt removal subtracting from it

The structure of bone is such that its surface area is enormous From a functional point of view there are three surfaces in the skeleton

(i) A surface at which no gross change occurs (90 per cent of bone surface in a normal adult skeleton)

(ii) A surface upon which new bone is being deposited

(iii) A surface from which bone is being resorbed

This bone mass is surrounded by body fluids which contain calcium and phosphorus

BONE FORMATION At the site of bone formation is found a layer of osteoid matrix covered by a thin layer of cells each of which has a single nucleus These cells are called osteoblasts and are thought to possess two functions

(i) To lay down the matrix in apposition to the already calcified bone
and
(ii) To elaborate the enzyme alkaline phosphatase

Normal osteoblastic activity requires adequate concentrations of ascorbic acid The bone salts in the form of hydroxyapatites are deposited in the osteoid tissue and may be seen as fine specks on the inner edges of the osteoid seams In addition osteoblastic activity is dependent to a high degree upon the wear and tear of normal body activity The concept of the stimulating effect upon osteoblastic activity exerted by the normal mechanical stress and strain of everyday life is fundamental to the understanding of the metabolism of bone in health and disease It was pointed out earlier in this chapter that the human skeleton is not a static body fixture but is constantly changing One of the important factors which promotes this ceaseless metabolic and structural moulding of bone is the continued effect of stress and strain When an invalid is confined to bed for long periods the absence of the usual mechanical stresses leads to a great decline in osteoblastic activity

During the formation of bone mineral deposition follows the elaboration of the organic matrix by osteoblasts Bone crystals are then formed within the cement substance their alignment being directed by the orientation of the collagen fibres (Fig 56) The cement substance of bone is capable of binding calcium and it has been suggested that it owes this property to the presence of chondroitin sulphate These observations indicate that the cement substance and the collagen of bone play an important part in the events associated with the deposition of bone

A number of other cations (e g sodium and magnesium) and anions (e g carbonate and citrate) are concentrated in bone but their respective roles in bone formation have not been elucidated Some of these ions may be adsorbed onto the surface of bone rather than incorporated into its crystalline structure

BONE RESORPTION At the surface where bone resorption takes place are found large multinucleated giant cells called osteoclasts Concerning the mechanism of bone resorption two theories

have been elaborated and as yet conclusive evidence has not been available to establish which of these is correct

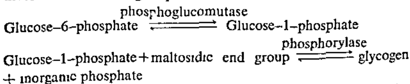
1) The removal of bone is governed by physico-chemical laws which control the equilibrium between the bone and the fluid which surrounds it The osteoclasts are foreign body giant cells respon sible for clearing debris

2) Osteoclasts by their own vital activity bring about the dissolu tion of bone

FACTORS AFFECTING CALCIUM AND PHOSPHORUS METABOLISM

1 *ALKALINE PHOSPHATASE* Since the body fluids bathe both the active surfaces of bone some explanation must be given for the deposition on one surface occurring pari passu with resorp- tion from the other One factor appears to be a local increase in the concentration of phosphate ions at surfaces where deposition is occurring This in turn results from the activity of alkaline phos phatase secreted by osteoblasts This enzyme has the property of splitting inorganic phosphate away from organic phosphate com pounds The level of serum alkaline phosphatase may be taken as *an index of osteoblastic activity in the absence of such conditions* as liver failure or obstructive jaundice

2 *PHOSPHORYLASE* Gutman and Gutman have demon strated the presence of the enzyme phosphorylase in growing car tilage and in bone but not in articular cartilage These workers have suggested that this enzyme may be important as a source of phosphorus in the formation of bone The polysaccharide glyco gen is found in cartilage before this tissue is subjected to calcifica tion and this store of glycogen is depleted during the process of ossification It is known moreover that the synthesis *of glycogen* involves the following steps —



It seems highly probable that phosphorylase can promote the uptake of phosphate by glycogen with the formation of glucose 1 phosphate and finally of glucose 6 phosphate i e the reverse of the above reactions The phosphate of glucose 6 phosphate would then become available for bone formation This theory overcomes one serious objection to the idea that alkaline phosphatase is the

principal factor in providing phosphate for calcification, namely that there is very little organic phosphorus in plasma (and hence in interstitial fluid) upon which phosphatase could act On the other hand phosphoric esters formed during the breakdown of glycogen (under the influence of phosphorylase) could provide sufficient phosphorus for the process of calcification

3 *PARATHYROID HORMONE* Parathyroid hormone when administered to animals without parathyroid tissue produces the following metabolic changes in the following order

1) An increase in the excretion of phosphorus in the urine
2) A fall in the serum inorganic phosphorus level
3) An increase in the serum calcium
4) An increase in the excretion of calcium in the urine

There are two theories which attempt to explain these changes One school believes that the hormone acts directly upon bone to effect its dissolution and that changes in calcium and phosphorus follow the changes in bone This dissolution is carried out by the osteoclasts which are stimulated by the hormone

Members of the other school believe that parathyroid hormone *in some way alters the phosphate dissolved in body fluids in such* a way as to make it more readily excreted by the kidney or that the hormone reduces tubular reabsorption of phosphate as the result of a direct effect upon the renal tubule The consequent loss of phosphate in the urine so alters the calcium phosphorus equilibrium of the blood as to stimulate the resorption of calcium and phosphorus from the bone resorbing surfaces

Some workers have tried to reconcile these views by suggesting that parathyroid hormone has two actions—to stimulate the excretion of phosphorus and to facilitate the resorption of bone both as primary actions Yet another school has postulated the existence of two hormones one stimulating phosphorus excretion and one acting upon bone in such a way as to promote resorption

In addition to these important changes parathyroid hormone raises the glomerular filtration rate but the significance of this observation remains obscure

4 *CALCIFEROL* (Vitamin D) Calciferol has two important actions

(i) It stimulates the absorption of calcium from the small intestine and therefore raises the serum calcium
(ii) It increases the rate of excretion of phosphorus in the urine

These two actions are quite independent of one another their relative importance and their sequelae depend upon the state of the parathyroid glands In the presence of normal parathyroid tissue the first effect overshadows the second The rise in serum calcium depresses parathyroid activity and hence the excretion of phosphorus falls The result is a rise in the serum levels of both calcium and phosphorus which leads to a decline in bone resorption and a return of serum calcium and phosphorus to normal On the other hand in individuals who lack parathyroid tissue the second action of calciferol becomes more apparent

5 *DIHYDROTACHYSTEROL* (AT_{10}) When AT_{10} is given to an individual without parathyroid tissue, a marked increase in the excretion of phosphorus occurs and is followed by a fall in serum phosphorus The absorption of phosphorus from the bowel is slightly increased while calcium absorption is moderately increased The result is a marked fall in serum phosphorus and a slight rise in serum calcium These changes lead to a fall in the solubility product and an increase in the resorption of inorganic salts from bone In the presence of normal parathyroid tissue the rise in serum calcium depresses parathyroid activity which results in a decrease in the rate of excretion of phosphorus and therefore a rise in serum phosphorus level This in turn causes a decrease in bone resorption and tends to lower the serum calcium and phosphorus levels

It can be seen that parathyroid hormone stimulates the excretion of phosphorus and may perhaps encourage the resorption of bone by some direct effect not yet understood it also causes a slight increase in the absorption of calcium from the bowel Calciferol increases the absorption of calcium from the bowel and stimulates the excretion of phosphorus but the effect on calcium absorption is more marked than that on phosphorus excretion Dihydrotachysterol on the other hand stimulates phosphorus excretion and has only a moderate effect upon the absorption of calcium from the bowel The rise in serum calcium due to calciferol results from increased absorption from the bowel the rise in serum calcium due to dihydrotachysterol is principally the result of resorption of bone In short dihydrotachysterol is intermediate in its action between parathyroid hormone and calciferol but it more nearly resembles the former However it should be pointed out that recent studies suggest that the chief difference between dihydrotachysterol and calciferol is quantitative rather than qualitative

TABLE I

The relative effects of calciferol dihydrotachysterol and parathyroid hormone upon the intestinal absorption of calcium and the urinary excretion of phosphorus

	Calcium absorption	*Phosphorus excretion*
Calciferol	++++	++
Dihydrotachysterol	++	+++
Parathyroid hormone	+	++++

PARATHYROID GLANDS

Embryology

The four glands are derived from the dorsal wings of the third and fourth pharyngeal pouches They are set free from the parental pouches during the seventh week of intrauterine life and begin to migrate in a caudal direction The pair from the third pouch descend further because they become attached to the developing thymus which brings them into a more caudal position than that of their fellows

Histology

The parathyroid gland is composed of densely packed groups of cells which are sometimes arranged in cords Between the cells a rich network of sinusoidal capillaries exists Two main types of epithelial cells are to be seen—the principal and the oxyphil cells The principal or chief cells are characterised by a large vesicular nucleus and pale cytoplasm The oxyphil cells may be arranged in groups and their cytoplasm contains granules which stain intensely with acid dyes

Function

The only known function of the parathyroid glands is to produce a hormone which plays a vital role in the metabolism of calcium and of phosphorus There is no direct evidence for the existence of two hormones and beyond the fact that parathyroid hormone is a protein little is known of its chemistry

Control

Parathyroid activity is controlled by the level of ionized calcium in the serum At least this is the only logical conclusion which can be drawn from present day experimental evidence Changes in the level of serum inorganic phosphate affect the solubility product of calcium and phosphorus and in this way influence the serum level of calcium but it should be clearly understood that the influence of phosphorus on parathyroid activity is indirect In short the parathyroid glands respond directly to the tissue requirements of calcium and indirectly to those of phosphorus A fall in serum calcium stimulates the secretion of parathyroid hormone while a rise in serum calcium exerts the opposite effect

FACTORS WHICH INFLUENCE SERUM CALCIUM AND PHOSPHORUS LEVELS

A number of factors are known to influence the levels of serum calcium and phosphorus but the important fact is that whenever serum calcium changes the activity of the parathyroid glands changes and this will in turn affect the rate at which phosphorus is lost in the urine and the rate of bone resorption Thus a fall in serum calcium leads to increased parathyroid activity and so to an increased urinary excretion of phosphorus Increased phosphorus excretion leads to a fall in serum phosphorus a fall in solubility product and this in turn causes an increase in bone resorption which raises the level of serum calcium A rise in serum calcium causes the opposite of these effects which eventually conspire to lower the serum calcium

CALCIUM The serum calcium level varies proportionately with the concentration of plasma protein and the hydrogen ion concentration of the plasma inversely with the concentration of serum inorganic phosphorus In addition the level of serum calcium is the outcome of the metabolism of calcium which includes

(i) The rate of absorption of calcium from the bowel

(ii) The rate of resorption of calcium from bone

(iii) The rate of deposition of calcium in bone

(iv) The rate of excretion of calcium in the urine

Absorption The absorption of calcium may be deficient when the diet is low in calcium when the intake of vitamin D is inadequate or when disease of the bowel interferes with absorption If the absorption of calcium be below the body requirements the

serum level of calcium may fall Excessive absorption of calcium may result from a high intake overdosage of vitamin D and rarely with diets low in fat The increased absorption of calcium is corrected by increased urinary excretion and the serum level remains normal However serum calcium levels may be raised as the result of an excessive intake of vitamin D

Resorption When the resorption of bone is inadequate the serum calcium falls This state of affairs occurs in hypoparathyroidism Excessive resorption of bone may exceed the capacity of the kidneys to excrete calcium and a rise in serum calcium results this occurs in hyperparathyroidism

Deposition Inadequate deposition of calcium in bone usually leaves the serum calcium level unaffected However the serum calcium may rise when excessive bone formation is suddenly arrested This situation may occur when growing children are immobilized and their bones no longer subjected to stress and strain By contrast excessive deposition of calcium causes a fall in serum calcium levels—this is seen when hyperparathyroidism is arrested by operation

Excretion The excretion of calcium is usually such as to favour the maintenance of normal serum calcium levels However in chronic renal disease the excretion of calcium may fall and in certain selective defects of renal tubular function the excretion rises

PHOSPHORUS Among the influences which may conspire to alter the level of serum phosphorus are the following

(i) The rate of absorption of phosphorus from the intestine
(ii) The rate of removal of phosphorus from the storage depots
(iii) The rate of deposition of phosphorus in the storage depots
(iv) The rate of excretion of phosphorus in the urine

The presence of phosphorus in glycogen intracellular fluid and bone means that serum levels are more easily maintained than those of calcium

Absorption Insufficient absorption of phosphorus tends to produce low serum levels This occurs in diets low in phosphorus or high in calcium or when the diet is rich in certain metals such as aluminium and magnesium Excessive absorption of phosphorus does not usually affect the serum phosphorus because compensatory changes occur Diets low in calcium or high in fat content may lead to such increased absorption

Resorption Resorption of phosphorus from bone is defective in hypoparathyroidism but this is offset by a fall in urinary

excretion so that serum phosphorus levels do not fall but in fact rise Phosphorus resorption from bone may be excessive in certain destructive diseases of bone, and these conditions may be associated with a raised serum phosphorus

Deposition The deposition of phosphorus in bone may be deficient when excessive bone formation is suddenly arrested or it may be excessive when hyperparathyroidism is arrested by operation Under these conditions the behaviour of serum phosphorus levels is variable but they frequently remain normal

Excretion Inadequate excretion of phosphorus in the urine may occur in pyelonephritis and under conditions of excessive resorption of bone the serum phosphorus rises Excessive urinary excretion may result in low serum levels in diseases of the kidney which interfere with tubular reabsorption of phosphorus, in low renal threshold and in hyperparathyroidism

One of the effects which results from the administration of growth hormone is a raised serum level of inorganic phosphorus The hormone causes retention of phosphorus without affecting calcium balance This is reflected in high serum phosphorus levels in growing children and patients with acromegaly

DISEASES OF THE PARATHYROID GLANDS

There are two major clinical syndromes which result from parathyroid disease—hypoparathyroidism and hyperparathyroidism These two diseases illustrate the physiology of the parathyroid glands so aptly that they are mentioned here

HYPOPARATHYROIDISM The parathyroid glands are sometimes inadvertently removed during operations on the thyroid gland In the absence of active parathyroid tissue the excretion of phosphorus in the urine falls and its level in the serum rises This produces a rise in the solubility product (Ca x P) and leads to a decline in the rate of bone resorption In turn this leads to a fall in serum calcium and so to a fall in urinary calcium The increase in solubility product in the blood may produce the deposition of calcium phosphate in such tissues as the lens and to an increase in the density of bone The fall in serum calcium results in increased neuromuscular excitability and tetany In addition to a fall in phosphorus excretion lack of parathyroid hormone is associated with a decline in the rate of bone resorption due to failure of direct action upon the skeleton This second or direct action of the hormone upon bone is thought to be the result of stimulation of osteoclasts

TETANY The word tetany is derived from the Latin tetanus meaning to stretch and is applied to attacks of muscular spasm affecting chiefly the hands and feet In its mildest form this amounts to no more than a feeling of stiffness in the hands while at its severest the larynx is affected and epileptiform convulsions occur Normal neuromuscular co ordination depends among other things upon a correct balance of certain ions in the blood A fall in serum calcium or an increase in the alkaline salts of the blood upset the balance and stimulate neuromuscular excitability It has been suggested that alkalosis may produce tetany by causing a fall in the amount of ionized calcium while the total serum calcium re mains normal The following list includes the important causes of tetany —

1) *Low Serum Calcium*
   - (i) Hypoparathyroidism
   - (ii) Deficient intake of calcium
   - (iii) Deficient vitamin D in the diet
   - (iv) Diseases of the bowel which interfere with the absorption of calcium
   - (v) Increased demand for calcium in pregnancy and lactation

2) *Alkalosis*
   - (i) Hyperpnoea—resulting from hysterical over breathing
   - (ii) Excessive ingestion of alkali
   - (iii) High intestinal obstruction

HYPERPARATHYROIDISM This condition results usually from a tumour of one or more parathyroid glands The glands pro duce an excess of parathyroid hormone which increases the urinary excretion of phosphorus and lowers the serum phosphate level This leads to a fall in the solubility product (Ca x P) and so to an increase in the resorption of bone unless it be that the intake of calcium and vitamin D is of such an order that the solubility product can be put right without interference with bone structure When bone resorption is stimulated a characteristic clinical and radiological picture results As more calcium continues to reach the blood more is excreted in the urine The serum calcium rises above normal levels and the glomerular filtrate becomes saturated with calcium salts which may precipitate out in the form of stones As a result cases of hyperparathyroidism may show bone disease renal disease or both some cases show only biochemical changes without bone or renal disease

REFERENCES

Instead of the usual reference numbers throughout the text of this chapter it has been thought more suitable to collect a small group of references under general headings The whole subject of parathyroid physiology has been beautifully and critically reviewed in "The Parathyroid Glands and Metabolic Bone Disease" Albright F and Reifenstein E.C Jr Williams & Wilkins Baltimore 1948

The Albright school have also published numerous papers dealing with special aspects of the subject including

Albright F J A M A 117 527 1941
Reifenstein E C Jr M Clin N A Boston Number *28 132 1944*
Albright F and Ellsworth R J Clin Invest 7 183 1939
Albright F et al J Clin Invest 11 411 1932

Another school of thought states its case in

Thompson D L and Collip J B Physiol Rev 12 309 1932
Collip J B et al *Brit J Exper Path 15 335 1934* and again
McLean F C & Bloom W Science 85 25 1937

The chemistry of bone and some biochemical aspects of ossification are found in

Roseberry H H et al J Biol Chem 81 479 1931
Logan M A Physiol Rev 20 525 1940

ADDENDUM

Recent experimental work has thrown considerable doubt upon the theory that calcification involves the precipitation of some phosphate of calcium *when the concentration of these ions exceeds their solubility in extracellular* fluid It now seems more likely that calcification is the result of step wise crystallization rather than precipitation and that a specific surface acts as a template or seed upon which crystallization takes place Such a surface would consist of specific groups in the collagen fibre molecules which would bind either calcium or phosphorus in a spatial configuration corresponding to the bone salt crystal lattice

Other experiments have done much to support the theory of a direct action of parathyroid hormone upon bone Resorption of bone involves the conversion of its three major constituents into substances which are soluble and can be transferred to the fluids of the body

1 The ground substance of bone contains polysaccharides which could be made soluble by a change in the size of their polymer molecules The enzyme hyaluronidase and the hormone of the parathyroid glands have been suggested as agents capable of effecting such a change

2 The collagen of bone is a protein which can be made soluble at the temperature and pH of body fluids by the action of certain proteolytic enzymes

3 The removal of the mineral component of bone has always been difficult to explain because it has been assumed that this process could only occur *in a highly acid medium The present* widespread use of chelating substances (chele meaning claw) in the decalcification of bone for histological preparations suggests a way out of this dilemma Chelating agents are capable of ring formation by co ordination with an unshared pair of electrons The ring so formed is poorly dissociated and the unshared electrons may be those of a metallic ion—in this case calcium Chelation can occur in neutral or alkaline solutions Proof is still lacking for the occurrence of these mechanisms *of bone resorption within the body but it* seems likely that parathyroid

hormone exerts a direct effect upon bone and brings about destruction of all three of its major components the above observations are suggested as possible mechanisms to explain this destruction

Hand in hand with such ideas concerning bone resorption a double mechanism for the control of serum calcium has been formulated Firstly a simple equilibrium exists between the labile fraction of bone mineral (amounting to about 5 % of the total mineral) and the serum calcium this equilibrium is independent of parathyroid control Such a mechanism will sustain plasma calcium levels of 7 mgm per 100 ml The second mechanism is necessary if serum calcium is to remain at the normal level of 10 mgm per 100 ml and this involves a feedback mechanism regulated by the parathyroid glands a fall in serum calcium exerts a direct stimulating influence upon these glands This stimulation results in release of parathyroid hormone which causes resorption of both the mineral and organic components of bone in this way restoring the serum calcium to its normal level

CHAPTER XII

ENDOCRINE FACTORS IN GROWTH, MATURATION AND DECLINE

Although the newborn infant has reached a stage in its development which is adequate for independent existence those processes of growth and differentiation initiated in foetal life must continue if maturity is to be attained Among the factors which control this *post natal development the activity of the endocrine glands is* important These glands are responsible for sexual maturation and play a vital part in somatic growth even before birth there is some evidence to show that the endocrine glands are active On the other hand after maturity has been reached there eventually comes a time when reproductive life draws to a close and the endocrine glands are called upon to establish a new balance in which sexual activity plays no part

Endocrine Activity Before Birth

Good evidence is now at hand to show that the foetal testis is not concerned only with its own morphogenesis but that it influences the development of the genital ducts and the external genitalia It would appear that the influence of the testis over the developing genital ducts is mediated through a secretion However this influence is chiefly if not entirely directed towards the homolateral duct and failure of development (or removal) of one testis allows the duct on the same side to develop in a female direction By inference it has been suggested that the foetal ovary produces no secretion to influence genital duct development which follows a female pattern unless otherwise directed by testicular activity Beyond these fundamental observations nothing is known of the nature of the testicular secretion concerned and although the Leydig cells at birth appear to show histological signs of activity nothing further can be said concerning the behaviour of the foetal gonads

It is known that the thyroid adenohypophysis and the pancreatic islets of the foetus are active at various stages of intrauterine life and it may be that other endocrine structures play an import

ant part in foetal development However while these activities are at present engaging the interests of a number of workers it is not possible to give a systematic description of endocrine physiology in the foetus

The Influence of the Endocrine Glands Upon Somatic Growth and Development

Cellular growth is greatly influenced by endocrine activity but some growth occurs independently of hormones The anabolism of protein is the fundamental factor concerned in growth and this is reflected chemically by the retention of nitrogen within the body and clinically by increase in skeletal length It must be borne in mind however that increase in height is only one aspect of growth and the fact that this increase is self evident and readily subjected to measurement should not cause growth in other dimensions to be overlooked

The endocrine control of growth may be looked upon as the outcome of a balance between those hormones which promote growth on the one hand and those which oppose it upon the other The hormones which encourage growth are those which stimulate protein synthesis those opposing growth are the hormones which are protein catabolic or anti anabolic The distinction between protein catabolism and protein anti anabolism is worthy of note It is probable that glucocorticoids for example do not so much destroy existing protein as favour the diversion of amino acids away from protein synthesis towards the synthesis of carbohydrate

The balance between protein anabolism and protein catabolism like the balance between the respective hormones which direct these activities is not constant throughout life Childhood is a period of powerful protein anabolic activity during which the hormones controlling this function gain ascendance over those which oppose them During adult life the two activities of protein synthesis and protein destruction reach a state of equilibrium and in old age the bones and other tissues reluctantly release some of their protein because the balance finally turns in favour of protein destruction The equilibrium of adult life must not be looked upon as indicating that protein metabolism is at a standstill on the contrary a considerable turnover of nitrogen by the body indicates that this is not the case This simply means that in the absence of disease anabolism and catabolism become virtually equal

The hormones which favour protein synthesis (and therefore growth) include —

1 Thyroid hormone
2 Growth hormone
3 Androgens
4 Insulin

The only hormones known to exert a destructive influence upon body protein are the glucocorticoids

THYROID HORMONE Thyroid hormone begins to exert its influence upon growth before birth It is essential to the metamorphosis of lower vertebrates and to the attainment of the adult size and bodily proportions of man The hormone is also essential for the full action of growth hormone the two being synergistic in their effect upon growth

Thyroid hormone also assists in the ossification of cartilage and the growth of the skeleton the eruption of the teeth the development of the brain and in bringing about the mature facial bone proportions of the adult That the hormone exerts these effects is shown by abnormalities seen in children born without normal thyroid tissue Under these circumstances bone and dental development are inadequate and abnormal children affected in this way are mentally retarded and their facial proportions continue to show the flat roundness of the newborn face

GROWTH HORMONE It seems likely that growth hormone makes its first appearance later than thyroid hormone it does not appear to play an important part in the growth which takes place during the first few years of life Growth hormone is the most powerful protein anabolic hormone and although much remains to be learnt about the exact way in which it promotes growth experimental evidence suggests that it acts directly upon the tissues stimulating protein synthesis from the amino acid pool of the body

ANDROGENS The growth spurt and muscular development seen during adolescence in both sexes are partly due to the action of adrenocortical androgens These hormones stimulate protein synthesis from the time of their first appearance at puberty In the male testicular androgens emphasise and elaborate the anabolic action of adrenocortical hormones The quantitative and qualitative differences between the androgens of boys and those of girls partly explain the better muscular development and heavier physique of the former

INSULIN Insulin is synergistic with the protein anabolic effects of growth hormone In addition insulin is known to exert a direct protein anabolic effect in its own right It has been shown

for example that insulin is capable of maintaining the growth of hypophysectomised animals It is probable however that the action of insulin upon protein metabolism is quantitatively less important than that of other protein anabolic hormones

GLUCOCORTICOIDS The glucocorticoids (chiefly cortisol or hydrocortisone) are the principal humoral opponents of protein anabolism and inhibit the incorporation of amino acids into tissue proteins especially in connective and lymphoid tissue In fact these hormones favour the diversion of amino acids towards the synthesis of carbohydrate Large doses of glucocorticoids adminis tered to growing children have been shown to impede their rate of growth

Fusion of epiphyses Cessation of linear growth is finally deter mined by fusion of the epiphyses of long bones Between the epiphysis and the diaphysis of a growing long bone there exists a plate of cartilage this is a temporary structure which enables longitudinal growth of the bone to take place This plate grows by multiplication of its cells and is continually replaced from both its epiphysial and diaphysial aspects by bone As a result of these two processes (cell multiplication and replacement by bone) proceeding at the same rate the plate of cartilage maintains a fairly constant thickness while the shaft of the bone increases in length Ultimately proliferation of the cartilage cells ceases and the entire plate becomes replaced by bone In this way the epiphy-sis unites with the diaphysis and the longitudinal growth of the bone comes to a close this process is referred to as the union or fusion of the epiphysis

The mechanism of this fusion is complex but it seems likely that the sex hormones play a major role Oestrogens and andro gens are both capable of closing epiphyses especially the former It has been suggested that these hormones bring about closure of the epiphyses by affecting the secretion of growth hormone It is more generally believed however that oestrogens in women and androgens in men are largely effective in this respect as the result of direct action upon bone

Apart from the limitations imposed upon linear growth by closure of the epiphyses the final size attained by an individual will also depend upon the inherent capacity of the tissues to re spond to protein anabolic hormones (especially growth hormone) Among the factors which govern this capacity to respond to these hormones genetic influences are important Eventually the secretion of growth hormone is inhibited but how this inhibition

1 Thyroid hormone
2 Growth hormone
3 Androgens
4 *Insulin*

The only hormones known to exert a destructive influence upon body protein are the glucocorticoids

THYROID HORMONE Thyroid hormone begins to exert its influence upon growth before birth It is essential to the metamorphosis of lower vertebrates and to the attainment of the adult size and bodily proportions of man The hormone is also essential for the full action of growth hormone the two being synergistic in their effect upon growth

Thyroid hormone also assists in the ossification of cartilage and the growth of the skeleton the eruption of the teeth the development of the brain and in bringing about the mature facial bone proportions of the adult That the hormone exerts these effects is shown by abnormalities seen in children born without normal thyroid tissue Under these circumstances bone and dental development are inadequate and abnormal children affected in this way are mentally retarded and their facial proportions continue to show the flat roundness of the newborn face

GROWTH HORMONE It seems likely that growth hormone makes its first appearance later than thyroid hormone it does not appear to play an important part in the growth which takes place during the first few years of life Growth hormone is the most powerful protein anabolic hormone and although much remains to be learnt about the exact way in which it promotes growth experimental evidence suggests that it acts directly upon the tissues stimulating protein synthesis from the amino acid pool of the body

ANDROGENS The growth spurt and muscular development seen during adolescence in both sexes are partly due to the action of adrenocortical androgens These hormones stimulate protein synthesis from the time of their first appearance at puberty In the male testicular androgens emphasise and elaborate the anabolic action of adrenocortical hormones The quantitative and qualitative differences between the androgens of boys and those of girls partly explain the better muscular development and heavier physique of the former

INSULIN Insulin is synergistic with the protein anabolic effects of growth hormone In addition insulin is known to exert a direct protein anabolic effect in its own right It has been shown

for example that insulin is capable of maintaining the growth of hypophysectomised animals It is probable however that the action of insulin upon protein metabolism is quantitatively less important than that of other protein anabolic hormones

GLUCOCORTICOIDS The glucocorticoids (chiefly cortisol or hydrocortisone) are the principal humoral opponents of protein anabolism and inhibit the incorporation of amino acids into tissue proteins especially in connective and lymphoid tissue In fact these hormones favour the diversion of amino acids towards the synthesis of carbohydrate Large doses of glucocorticoids adminis tered to growing children have been shown to impede their rate of growth

Fusion of epiphyses Cessation of linear growth is finally deter mined by fusion of the epiphyses of long bones Between the epiphysis and the diaphysis of a growing long bone there exists a plate of cartilage this is a temporary structure which enables longitudinal growth of the bone to take place This plate grows by multiplication of its cells and is continually replaced from both its epiphysial and diaphysial aspects by bone As a result of these two processes (cell multiplication and replacement by bone) proceeding at the same rate the plate of cartilage maintains a fairly constant thickness while the shaft of the bone increases in length Ultimately proliferation of the cartilage cells ceases and the entire plate becomes replaced by bone In this way the epiphy sis unites with the diaphysis and the longitudinal growth of the bone comes to a close this process is referred to as the union or fusion of the epiphysis

The mechanism of this fusion is complex but it seems likely that the sex hormones play a major role Oestrogens and andro-gens are both capable of closing epiphyses especially the former It has been suggested that these hormones bring about closure of the epiphyses by affecting the secretion of growth hormone It is more generally believed however that oestrogens in women and androgens in men are largely effective in this respect as the result of direct action upon bone

Apart from the limitations imposed upon linear growth by closure of the epiphyses the final size attained by an individual will also depend upon the inherent capacity of the tissues to re spond to protein anabolic hormones (especially growth hormone) Among the factors which govern this capacity to respond to these hormones genetic influences are important Eventually the secretion of growth hormone is inhibited but how this inhibition

is brought about remains obscure It has been suggested that the sex hormones are capable of inhibiting the secretion of growth hormone but whatever may be the mechanism during adolescence the adenohypophysis directs its activity away from the secretion of growth hormone in favour of the production of gonadotrophins

The Influence of the Endocrine Glands Upon Sexual Development

PUBERTY The first event in the onset of puberty which has been observed is the secretion of gonadotrophic hormones by the adenohypophysis These hormones appear in the urine quite suddenly as though in response to a trigger mechanism rather than gradually appearing in increasing concentrations One can only speculate on the precipitating mechanism of this release It has been suggested that the hypothalamus is responsible for stimulating the pituitary to produce gonadotrophins especially luteinizing hormone Hereditary and genetic factors appear to play some part as revealed by family and racial studies of the onset of puberty Again it may be that the body or the endocrine glands themselves must attain a certain degree of maturity before the pituitary secretes gonadotrophins Certainly the onset of puberty seems to bear some relationship to the stage of development of the bones Finally it is possible that a certain maturity of the central nervous system may be required before the onset of puberty can take place Factors which in turn would influence the maturity of the tissues include thyroid hormone, androgenic hormones growth hormone and genetic factors However it must be said that at present the fundamental mechanism which sets in motion the changes of puberty is unknown

Soon after the appearance of gonadotrophins in urine an increase in the excretory products of androgenic hormones is observed in the urine of both sexes Again the mechanism which precipitates this androgenic activity is unknown Gradually however, the accessory sexual organs and the secondary sexual characteristics develop in response to the steroid hormones secreted by the adrenal cortex and gonads The relative importance of the adrenal cortex and the gonads in bringing about these changes probably differs in the two sexes In the male the development of the sexual organs results from the action of androgens and in this respect the role of cortical androgens is somewhat overshadowed by the activity of testicular hormones In the female oestrogens secreted by the ovary are supplemented by androgens

from the cortex and together these two groups of hormones induce the development of mature sexual organs and secondary sexual characteristics

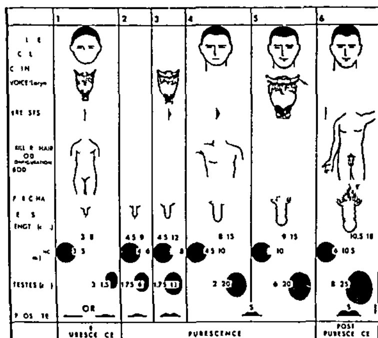


Fig 57 Stages of sexual development in the male (*Lawson Wilkins*)

SEXUAL DEVELOPMENT IN THE MALE (Table I and Fig 57) The first demonstrable change in boys at puberty is an increase in the size of the testis This is due to the direct action of gonadotrophins Luteinizing hormone stimulates the interstitial cells which respond by secreting androgens The concomitant maturation of the seminiferous tubules is due to the secretion of follicle stimulating hormone It is the development of the tubules which causes increase in the size of the testis since they constitute the bulk of the organ In boys an increase in urinary 17 ketosteroids occurs at the age of 8 or 9 years The androgens secreted by the adrenal cortex and testis bring about the following changes —

(i) Increased vascularity of the penis and scrotum

(ii) Thickening wrinkling and pigmentation of the skin of the scrotum

(iii) Increase in the size of the penis
(iv) Increase in the size of the prostate gland
(v) Appearance of pubic and axillary hair
(vi) Development of the larynx and deepening of the voice
(vii) Sebaceous gland activity and acne

TABLE I

Average Approximate Age and Sequence of Appearance of Sexual Characteristics in Both Sexes (Lawson Wilkins)

Age years	*Boys*	*Girls*
9 10		Growth of bony pelvis Budding of nipples
10-11	First growth of testes and penis	Budding of breasts Pubic hair
11 12	Prostatic activity	Changes in vaginal epithelium and the smear Growth of external and internal genitalia
12 13	Pubic hair	Pigmentation of nipples Mammae filling in
13 14	Rapid growth of testes and penis Subareolar node of nipples	Axillary hair Menarche (Average 13½ yrs Range 9 17 yrs) Menstruation may be anovulatory for first few years
14 15	Axillary hair Down on upper lip Voice change	Earliest normal pregnancies
15 16	Mature spermatozoa (Aver 15 yrs range 11½-17 yrs)	Acne Deepening of voice
16 17	Facial and body hair Acne	Arrest of skeletal growth
21	Arrest of skeletal growth	

SEXUAL DEVELOPMENT IN THE FEMALE (Table I) The ovary begins to secrete oestrogens under the influence of pituitary gonadotrophins These produce the first evidence of puberty in the female namely increase in the size of the nipple and areola together with pigmentation of these structures Oestrogens also cause —

(i) Development of the stroma and ducts of the breast
(ii) Development of the labia minora and vulva
(iii) Increase in volume of the vagina uterus and tubes
(iv) The appearance of stratified squamous epithelium in the vagina which becomes thrown into rugae

Adrenocortical androgens assist in the evolution of the signs of puberty in the following ways

(i) Development of pubic and axillary hair
(ii) The development of the clitoris and labia majora
(iii) Stimulation of sebaceous activity and the appearance of acne

Cyclic changes in the secretion of oestrogens bring about oestrogen withdrawal bleeding approximately every four weeks Later ovulation occurs and is followed by the formation of a corpus luteum together with the secretion of progesterone This hormone brings about the occurrence of fertile menstrual cycles and further development of the breast Generally the secretion of oestrogens and adrenocortical androgens proceed side by side so that the events of puberty succeed one another in an orderly pattern In some normal girls however one group of hormones may be secreted earlier than the other and a considerable period of time may thus separate the appearance of oestrogenic and androgenic effects The sequence of events which go to make up normal puberty are shown in Table I However it should be clearly understood that great variation is seen in the time relationships between the various features of puberty in normal individuals no hard and fast rules can be laid down and the events of puberty do not keep to a strict timetable The onset of puberty in normal children may occur between the ages of 9 and 17 years and the individual events may follow one another in quick succession or the whole process may be drawn out taking many years to complete Among the factors which determine the onset and rate of these changes are variations in the relative concentrations of the trophic hormones of the pituitary and variations in the response of target organs

INDICES OF SOMATIC MATURATION Between birth and the attainment of adult development the individual processes of somatic maturation occur in a definite sequence so that in normal individuals it is possible to predict the time of appearance of these changes Sometimes delay or precocity occurs in one or more aspects of maturation and it is possible to measure the extent of such aberrations by comparing their time of observed onset with that expected in normal individuals In this way a number of indices of maturation can be derived which offer a useful clinical guide to the extent of any delay or acceleration of maturation as a whole, or of any part of its several aspects As in the case of

the sequence of events in the evolution of puberty however, the rate of somatic maturation is subject to variation in normal individuals

1) *Body Proportions* Between birth and maturity not only does the height of an individual increase but the limbs become longer in proportion to the total body length (Fig 58) At the time of maturity the span or distance between the finger tips of the outstretched arms is equal to the height, and the measurement from the vertex to the upper border of the symphysis pubis, with the subject standing is equal to the measurement from the symphysis to the floor By comparing the height with span and upper and lower body segments with expected normals for age sex and race one can determine the skeletal maturity of an individual

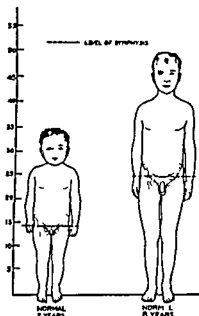


Fig 58 The figures of normal boys aged two and eight years showing the change in the ratio of the upper and lower segments measured from the symphysis pubis At birth the ratio is 1 7 and at ten years 1 0 (*After Lawson Wilkins*)

2) *X Ray Examination of Bones* The appearance and union of the various epiphysial centres of ossification normally follow a fairly definite pattern from birth to maturity The stage of development of this pattern at a given moment is called the bone age and is determined by the presence or absence of calcification in the various cartilages which normally ossify at specific ages Certain

changes in the shape of the bones near joints occur with some regularity and such changes form the basis of a more accurate but more complex means of studying the maturity of the skeleton

3) *Dental Development* As in the case of epiphysial maturation the teeth develop and erupt in accordance with a definite schedule It is therefore possible to compare the state of dental development of an individual with that expected for his age this can be expressed as the dental age

4) *Intelligence* It is possible to assess intelligence in relation to age the result being expressed as an intelligence quotient or as the mental age of an individual

5) *Maturation of the Face* During infancy the face undergoes certain structural changes which involve especially the bridge of the nose The nose and jaw become longer, which together with other changes give the face a more mature appearance Although these changes cannot be subjected to accurate measurement delay in maturation of the facial appearance is sometimes an important sign of disease of endocrine structures

THE CLIMACTERIC

The end of a woman s reproductive life is usually a gradual process It commonly extends over a period of one to three years and usually begins between the ages of 45 and 50 The term climacteric (meaning rung of a ladder) is appropriate for this period of transition and is to be preferred to the word menopause which refers only to the cessation of menstruation However the word menopause may be used to refer to this one aspect of the climacteric but the two words should not be looked upon as synonymous

As in the case of puberty a wide variation is seen in the climacteric of women It may vary from an abrupt symptom free cessation of menstruation on the one hand to a protracted period of increasing irregularity of menstruation on the other In addition to the cessation of bleeding ovulation also stops but these two changes are not necessarily synchronous Most commonly ovulation stops before bleeding with the result that the last cycles of a woman s reproductive life like the first are anovulatory Rarely menstruation stops before ovulation

The essential endocrine basis of the climacteric is a decline in the secretion of oestrogens by the ovary This leads to a disturbance in the balance of pituitary ovarian relations and so to the cessation of ovulation What factors are responsible for limiting the

functional life of the ovary cannot be stated The relationship between the ovary and the adenohypophysis cannot now return to that which prevailed before puberty because lack of oestrogen secretion now stimulates the production of excess FSH The same train of events follows the destruction or removal of the adult ovary at any stage during the period of established reproductive life

Failure of oestrogen secretion leads to regression of the accessory genital organs and of the secondary sexual characteristics The uterus gradually decreases in size and the endometrium undergoes atrophy The volume of the vagina diminishes and glycogen disappears from the thinning mucosa which deprives the organ of its acid reaction The breasts become flabby and soft while their parenchymatous tissue atrophies The skin becomes less smooth and more wrinkled there is a tendency to put on weight (especially round the hips) and some loss of pubic and axillary hair occurs

In addition to these manifestations of oestrogen withdrawal there occur a train of vasomotor and psychic changes which are extremely variable in their manifestations and their intensity These changes include hot flushes palpitations irritability and headache

The most characteristic hormonal change to be observed at the climacteric is an excess of FSH secretion urinary levels may be raised to between 10 and 150 times the normal This increase in urinary gonadotrophins results from the loss of oestrogenic inhibition of the adenohypophysis (page 117) Urinary oestrogen levels fall to very low levels but do not entirely disappear, probably because some oestrogen is secreted by the adrenal cortex Pregnanediol almost entirely disappears from the urine after the climacteric while 17 ketosteroid excretion is usually unchanged

MALE CLIMACTERIC The end of reproductive life in men is even more gradual and more variable than in women It cannot be said that normal men pass through a physiological readjustment comparable with the female climacteric Some men however, appear to suffer the same symptoms and to show the same rise in the level of gonadotrophin excretion as those described in the case of women This state of affairs is associated with a relatively abrupt failure of testicular function which may justify the use of the term climacteric On the other hand the majority of men enter into a period of sexual decline which is so gradual that it passes without producing symptoms

After the climacteric there gradually appear in both sexes the relentless changes of old age Some of these adjustments concern the endocrine glands which eventually come to favour protein catabolism a state which is reflected by loss of body protein from bone and muscle However the changes responsible for the onset of senility are much more widespread than those observed in the endocrine glands at this time of life Although these changes are being studied at present it is not possible to account for the role of the endocrine glands in the processes of decline which characterise old age

REFERENCES

The endocrinology of growth and maturation is discussed in detail by Wilkins L in his Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence C C Thomas Illin 1950 and by Talbot N B et al in "Functional Endocrinology Harvard Mass 1952

CHAPTER XIII

THE NERVOUS CONTROL OF THE ENDOCRINE GLANDS

Introduction

The nervous system and the endocrine glands are the two great controlling organisations of vertebrate physiology and between these two systems there exists a reciprocal relationship The nervous system directly or indirectly controls the adenohypophysis *the neurohypophysis ovaries testes thyroid adrenal cortex and* medulla On the other hand the hormones of these glands, or changes which they produce in the tissues upon which they act react upon the nervous system to produce a variety of neurological changes It is clear moreover that the nervous system is largely responsible for correlating endocrine activity with that of the other systems of the body and with the changing requirements of the organism resulting from environmental changes

In the case of the neurohypophysis and of the adrenal medulla such nervous control is easily understood, since both structures are *richly supplied with nerves* The neurohypophysis is *directly* connected with the hypothalamus by the hypothalamo-hypophysial tracts of nerve fibres while the adrenal medulla is connected to the hypothalamus by descending tracts in the brain stem and spinal cord and by the final nerve supply which passes in the splanchnic nerves and lumbar sympathetic chain It is otherwise in the case of the remaining endocrine glands These structures are clearly controlled in some way by the nervous system but they possess no more than poorly developed nerve supplies which are probably vasomotor in nature and not secretomotor

It is difficult then, to explain how the central nervous system controls the adenohypophysis the adrenal cortex *the thyroid* gland and the gonads However it is well known that the adeno hypophysis influences the function of the adrenal cortex the thyroid gland and the gonads by way of its trophic hormones If it can be established that the central nervous system controls the activity of the adenohypophysis, then it will be clear that the

remaining glands are indirectly subjected to nervous control through their association with the anterior pituitary gland

It will be shown that although the adenohypophysis possesses but a poorly developed nerve supply it has a most extraordinary vascular system which includes a system of portal vessels These portal vessels arise from capillary loops in the median eminence and give rise to sinusoidal capillaries in the pars distalis It is believed that the hypothalamus influences adenohypophysial function by means of these portal vessels In this way the anterior pituitary gland (and hence its target glands) are controlled by the central nervous system (Fig 1)

As for the other side of this neuroendocrinal reciprocity it may be said that the target hormones exert two main effects upon the central nervous system In the first place they control the rate of their own secretion by acting either directly upon the pituitary gland or upon the hypothalamus (Fig 1) It will turn out that the rate of secretion of a given trophic hormone by the pituitary gland is the sum of a number of factors one of which is the concentration in the blood of the hormone whose secretion it stimulates (Chapter VII) In the second place hormones affect the behaviour of man and animals this may be due in some instances to a direct action upon the nervous system

The Neural Control of the Adenohypophysis

The nerve supply to the adenohypophysis is poorly developed and appears inadequate to explain the influence of the nervous system upon the secretory activity of the gland On the other hand the blood supply of the adenohypophysis is marked by the presence of a well developed system of portal vessels and many workers now believe that these vessels play an important role in the relationship between the central nervous system and the adenohypophysis

NERVE SUPPLY OF THE ADENOHYPOPHYSIS The adenohypophysis receives a sympathetic nerve supply from the nerves surrounding the internal carotid artery This supply is distributed to the pars tuberalis and experimental evidence seems to indicate that it is not important in the control of the gland The adenohypophysis also receives a parasympathetic nerve supply but again there is no good evidence to show that this is important to the function of the gland

Finally the hypothalamus gives rise to the hypothalamo hypophysial tract which innervates the neurohypophysis A few fibres from this tract enter the pars tuberalis and the pars intermedia but it is doubtful if any reach the pars distalis (Fig 61)

BLOOD SUPPLY OF THE ADENOHYPOPHYSIS The adenohypophysis receives two distinct blood supplies, one is systemic and the other portal

Systemic Arterial An anterior and a posterior hypophysial artery arise from each internal carotid artery (Fig 59) The anterior vessel distributes branches to the pituitary stalk and gives off a substantial branch the artery of the trabecula which courses through the pars distalis without branching and supplies the lower part of the infundibular stem Each posterior hypothalamic artery divides into a medial and lateral branch (Fig 59) These branches anastomose with their fellows from the opposite side giving rise in this way to an arterial ring around the neural lobe Branches from this ring supply the neural lobe and part of the infundibular stem but neither the anterior nor the posterior hypophysial vessels supply the pars distalis

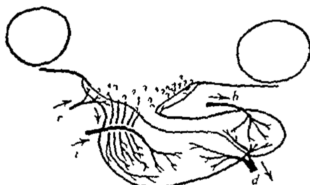


Fig 59 Diagram of a sagittal section through the pituitary gland of a rabbit illustrating the blood supply to the gland *a* anterior hypophysial artery *b* posterior hypophysial artery *c* twigs to the plexus of the pars tuberalis derived from the internal carotid and posterior communicating arteries *d* venous drainage (*G W Harris*)

Venous The venous drainage of the adenohypophysis is by short wide veins which empty directly into venous sinuses situated around the gland or inferiorly into the sphenoidal sinus (Fig 59)

Portal Small arterial twigs from the internal carotid and posterior communicating arteries run to supply a rich vascular plexus in the pars tuberalis of the adenohypophysis (Fig 59) From this plexus there arises a multitude of capillary loops or tufts which penetrates into the tissue of the median eminence and there comes into intimate contact with the nerve fibres of the hypothalamo-hypophysial tract These loops or tufts are collec

tively called the primary plexus of the hypophysial portal vessels The blood from the primary plexus empties into large portal trunks which lie mainly on the anterior surface of the pituitary stalk where they are visible to the naked eye It may now be accepted as proven that the direction of blood flow in this system of vessels is from the hypothalamus towards the pituitary

The portal trunks eventually break up into smaller branches within the pars distalis and end by anastomosing with the sinusoids which form such a characteristic feature of the histology of this structure and about which the secreting cells of the adenohy pophysis are disposed

Two features of this portal system of vessels are of interest In the first place these vessels show an extraordinary capacity for regeneration after they have been cut Following division of the pituitary stalk a capillary network from these vessels may even penetrate the interstices of a plug of cotton wool placed between the divided ends of the pituitary stalk and in this way the vascular connection between the capillary loops of the median eminence and the vessels of the pars distalis is re-established It is this capacity for regeneration which is largely responsible for the suc cessful function of pituitary grafts on the ventral surface of the brain after hypophysectomy Secondly the evolution of this sys tem of vessels from lower vertebrates to man shows a very delib-erate elaboration which reaches its highest development in pri mates This observation suggests that the hypophysial portal vessels play an important role in pituitary function and do not represent vestigial remnants left behind in the process of the evolution of the adenohypophysis

FUNCTION OF THE HYPOPHYSIAL PORTAL VESSELS

The appearance situation and the evolution of the hypophysial portal vessels all suggest that they constitute an important functional unit As the result of an exhaustive series of experi ments Harris and others have come to suggest that the portal vessels constitute an integral part of the normal pituitary gland and that it is through this system of vessels that the hypothalamus may regulate the rate of secretion of the adenohypophysis Harris elaborated this theory by studying the effect of hypothalamic stimulation upon the rate of release of the individual trophic hor mones of the adenohypophysis He developed a technique which made it possible to apply a measured electrical stimulus to a minute area within the brain without anaesthesia and with out handling the experimental animal In a preliminary opera tion a small coil is inserted under the scalp and an insulated

electrode attached to one end of this coil is passed through the skull and corpus callosum into some part of the hypothalamus or pituitary gland After recovery from the operation the tissue surrounding the electrode tip could be stimulated by holding a primary coil carrying an alternating current over the animal's head so that the coil under the scalp lies in an electromagnetic field The strength of the stimulus is easily regulated since it varies inversely as the distance between the two coils With this apparatus a small area of brain tissue could be stimulated without disturbing the animal in any way The approximate site of stimulation is shown in x rays of the skull while the exact position in each experiment is determined at autopsy Electrical stimulation of the hypothalamus produces no overt emotional response in these animals *The great advantage of this technique lies in the fact that* such stressor stimuli as restraint and anaesthesia are eliminated and the state of affairs prevailing before electrical stimulation is applied can be regarded as quiescent

These experiments indicate that stimulation of the hypothalamus promotes the secretion or release of trophic hormones by the anterior pituitary gland while stimulation of the gland itself is ineffective Now it is known that the pars distalis receives no more than a very scanty nerve supply from the hypothalamus Therefore in order to explain the influence exerted by the hypothalamus upon adenohypophysial function Harris has suggested that the nerve fibres of the hypothalamo hypophysial tract may liberate some humoral substance(s) into the capillaries of the primary plexus of the median eminence and that this substance is carried by the hypophysial portal vessels to the pars distalis where it may excite or inhibit the secretory activity of the cells of the pars distalis That such a conception is entirely feasible is shown by the intimate contact established between the nerve fibres and the capillary loops within the median eminence The loops consist of little more than endothelial cells and are not in close contact with nerve cells This suggests that if these loops have any function at all it must be related in some way to the intimate association they bear with the fibres of the hypothalamo hypophysial tract Final confirmation of this hypothesis remains to be established since evidence *for the existence of a chemical substance passing from* nerve fibres to capillary loops is still entirely circumstantial Extracts of tissue from the median eminence have not revealed the nature of such humoral substances while the action of adrenaline and acetylcholine do not suggest either an adrenergic or a cholinergic mechanism

The arrangement of the hypophysial portal vessels amongst themselves has further suggested a way in which the hypothalamus might influence the secretion of one trophic hormone rather than another The pars distalis appears to be capable of division into medial and lateral parts according to the distribution of the com ponent glandular cells The portal trunks are also clearly divisible into medial and lateral groups At the same time these trunks bear an end artery relationship with the different regions of the gland they supply In other words one group of portal trunks confines its attention to one part of the anterior pituitary gland It has been suggested therefore that medial and lateral nerve tracts within the median eminence may be related to medial and lateral portal tracts and these in turn to medial and lateral parts of the pars distalis (Fig 60) In this way it is conceivable that different nervous stimuli could affect different parts of the median eminence and in turn different parts of the pars distalis and so perhaps produce a different secretory response from the gland In this way anterior pituitary function could be regulated to meet the particular requirements of the body at a given time and would thereby be freed from the necessity of giving an all or nothing response to hypothalamic stimulation

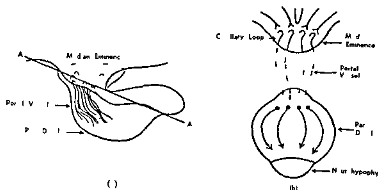


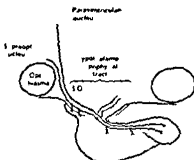
Fig 60 (*a*) Shows a diagrammatic section of the pituitary gland The line A — A indicates the plane of section which is seen from above in (*b*)

(*b*) Is strictly diagrammatic and is designed to illustrate the possibility that different groups of fibres from the hypothalamus affect the function of different groups of portal vessels which are in turn distributed to different segments of the adenohypophysis (*G W Harris*)

THE NEURAL CONTROL OF THE NEUROHYPOPHYSIS

The neural control of neurohypophysial function rests upon more direct evidence than that of the adenohypophysis It has been

shown in Chapter VIII that not only does the hypothalamus control the rate of release of neurohypophysial secretions into the general circulation but that the very secretions themselves are thought to arise from the neurones of the paraventricular and supraoptic nuclei It is further believed that these secretions are eventually delivered into the blood vessels of the neurohypophysis about which nerve fibres from the supraoptic and paraventricular nuclei end



S O H Supraoptico-hypophysial Tract

T H Tubero hypophysial Tract

Fig 61 Diagrammatic representation of the hypothalamo-neurohypophysial system The hypothalamo hypophysial tract is shown arising in the supraoptic and paraventricular nuclei and ending in the median eminence the infundibular stem and in the infundibular process (*G H Harris*)

Nerve Supply of the Neurohypophysis It should be recalled that the neurohypophysis includes the median eminence the infundibular stem, and the infundibular process these three parts together constituting a single gland Apart from a few sympathetic fibres from the carotid plexus which are vasomotor in their action the main nerve supply to the neurohypophysis is from the hypothalamo-hypophysial tract in the pituitary stalk This tract is divisible into two parts the supraoptico hypophysial tract which lies in the anterior wall of the pituitary stalk and the tubero-hypophysial tract in the posterior wall (Fig 61)

The supraoptico hypophysial tract arises from the supraoptic paraventricular and perhaps from other hypothalamic nuclei The tubero hypophysial tract arises from the central and posterior parts of the hypothalamus (i e from the ventral paraventricular nucleus and from scattered cells and nuclei in the tuberal region and the mammillary bodies) As the supraoptico hypophysial tract enters the median eminence it is very superficial and therefore vulnerable to disease processes The tubero hypophysial tract is smaller and composed of finer fibres than the supraoptico hypophysial tract (Fig 61)

Most of the fibres of these two tracts end in the neurohypophysis although a few enter the pars tuberalis and the pars intermedia of the adenohypophysis The hypothalamo hypophysial tract supplies all three divisions of the neurohypophysis The endings of these tracts may be secretory or secretomotor but they make extensive arborizations about blood vessels and it seems possible that they deliver their secretions directly into these vessels

BLOOD SUPPLY OF THE NEUROHYPOPHYSIS In spite of statements to the contrary the neurohypophysis is richly supplied with blood vessels which come from two sources

(i) The median eminence receives a blood supply from the primary plexus of hypophysial portal vessels This is derived from the plexus of the pars tuberalis and so from the internal carotid artery The same plexus also supplies the infundibular stem

(ii) The infundibular process receives a separate blood supply from the posterior hypophysial artery which gives rise to an arterial ring around the gland at the junction of the neural lobe and the pars distalis

The neurohypophysis should therefore be looked upon as a structure developed for the storage of hormones secreted by the hypothalamus The neurohypophysis is entirely controlled by its nerve connections with the hypothalamus which regulate the rate at which the stored hormones are released into the blood stream (Chapter VIII)

NERVE FIBRE CONNECTIONS OF THE HYPOTHALAMUS The vital role played by the hypothalamus in the control of endocrine activity makes the connections between this structure and other parts of the central nervous system a subject of great interest It is through the hypothalamus that the central nervous system influences endocrine activity and in this way the function of the endocrine glands is influenced bv a wide variety of changes arising both within and without the body

1 *Mammillary peduncle* This tract appears to convey afferent nerve impulses which are capable of affecting the secretion of ACTH and gonadotrophins It is poorly developed in man and requires further study before the importance of the sensory impulses which it conveys can be assessed

2 *Fornix* The fornix system conveys afferent fibres to the hypothalamus It arises from the hippocampus and ends in the nuclei of the mammillary body Although its exact function remains undetermined the fornix may be connected with the physiology of emotion

3 *Mammillo-thalamic tract* The fibres which go to make up this tract leave the medial mammillary nucleus to reach the anterior nuclei of the thalamus Through this tract a connecting link exists between the hypothalamus and the neocortex

4 *Mammillo tegmental tract* This tract arises from the medial mammary nucleus but its destination remains obscure

5 *Medial forebrain bundle* Fibres both enter and leave this bundle as it passes through the hypothalamus on its way from the olfactory area of the hemisphere to the tegmentum of the mid brain

6 *Periventricular system* This is a two-way path between the medially situated nuclei of the hypothalamus and the medial thalamic nuclei

7 *Diffuse descending system* These fibres leave the hypothalamus and descend in relays within the reticular formation of the brain stem to the spinal cord

8 *Neocortical connections* A good deal of physiological evidence can be produced to support the belief that connections exist between the neocortex and the hypothalamus Clark expressed this view when he stated that the greater part of the cortex of the frontal lobe may be regarded as a projection area of hypothalamic activity Apart from indirect connections established through the thalamus direct connections have been described between cortical areas 6 8 and 9 on the one hand and the mammillary nuclei of the hypothalamus on the other Other direct pathways have been described including nerve tracts to the centromedial hypothalamic nucleus which serves as an end station for orbital fibres

It will therefore be seen that the hypothalamus acts in the neural control of the endocrine glands as a focal point at which are gathered impulses from various parts of the central nervous system The hypothalamus responds to these influences by altering the function of the adenohypophysis as well as that of the neurohypophysis The first of these structures is affected by way of its vascular supply the second by way of its nerve supply The adenohypophysis relays this hypothalamic influence to other endocrine glands through the action of its trophic hormones

HORMONES AND BEHAVIOUR

Although it is clear that hormones affect animal and human behaviour it is not known exactly what effect each hormone exerts nor is it certain how these effects are brought about Experiments with certain animals which establish measurable acts of submis

sion and aggression have suggested that oestrogens are responsible for submissive behaviour and that androgens induce aggression For example a group of hens sharing the same pen establish what amounts to an order of seniority which is called a peck order One hen always eats first and attacks (or pecks) all the other hens The remaining animals organise themselves in such a way that a particular hen is pecked by those higher in peck order and attacks those below it If an androgenic hormone be administered to the most submissive animal it will ascend in peck order eventually reaching the top while oestrogens will cause the most aggressive hen to descend the scale These observations are consistent with the effects which these hormones produce in man but how such changes are effected cannot be said

Again the hormones prolactin and progesterone appear to stimulate the development of the maternal instinct Virgin female rats treated with prolactin begin to exhibit maternal behaviour towards young rats which they normally reject In women experiments have been performed to correlate changes in hormonal activity with behaviour The follicular phase of the menstrual cycle during which oestrogens are secreted is associated with gradually increasing heterosexual tension After ovulation a marked change in behaviour is recognised and a passive narcissistic role is adopted The subsequent premenstrual secretion of oestrogens is associated with the return of heterosexual orientation[1]

Diseases of the thyroid gland and of the adrenal cortex are frequently associated with disturbances of behaviour It is probable that the hormones secreted by these two glands influence behaviour but how and in what direction remain to be determined Adrenocortical extracts will prevent the occurrence of the experimental neurosis which follows the differential inhibition of a conditioned reflex For example if a conditioned reflex of salivation be established to the visual stimulus of a white square of paper followed by feeding the experimental animal can also be conditioned to distinguish the square from a similar circle which is not followed by feeding In this way the animal salivates when shown the square but gives no response to the circle If however the square be made more circular and the circle more nearly square a point is reached at which the animal is unable to distinguish one stimulus from the other and begins to exhibit neurotic behaviour Animals treated with adrenocortical extracts at first salivate in response to the doubtful stimulus but soon lose interest and ignore it In this way they avoid the experimental neurosis shown by untreated animals

3 *Mammillo-thalamic tract* The fibres which go to make up this tract leave the medial mammillary nucleus to reach the anterior nuclei of the thalamus Through this tract a connecting link exists between the hypothalamus and the neocortex

4 *Mammillo tegmental tract* This tract arises from the medial mammary nucleus but its destination remains obscure

5 *Medial forebrain bundle* Fibres both enter and leave this bundle as it passes through the hypothalamus on its way from the olfactory area of the hemisphere to the tegmentum of the mid brain

6 *Periventricular system* This is a two-way path between the medially situated nuclei of the hypothalamus and the medial thal amic nuclei

7 *Diffuse descending system* These fibres leave the hypoth alamus and descend in relays within the reticular formation of the brain stem to the spinal cord

8 *Neocortical connections* A good deal of physiological evi dence can be produced to support the belief that connections exist between the neocortex and the hypothalamus Clark expressed this view when he stated that the greater part of the cortex of the frontal lobe may be regarded as a projection area of hypothalamic activity Apart from indirect connections established through the thalamus direct connections have been described between cortical areas 6 8 and 9 on the one hand and the mammillary nuclei of the hypothalamus on the other Other direct pathways have been described including nerve tracts to the centromedial hypoth alamic nucleus which serves as an end station for orbital fibres

It will therefore be seen that the hypothalamus acts in the neural control of the endocrine glands as a focal point at which are gathered impulses from various parts of the central nervous system The hypothalamus responds to these influences by altering the function of the adenohypophysis as well as that of the neuro-hypophysis The first of these structures is affected by way of its vascular supply the second by way of its nerve supply The adeno-hypophysis relays this hypothalamic influence to other endocrine glands through the action of its trophic hormones

HORMONES AND BEHAVIOUR

Although it is clear that hormones affect animal and human behaviour it is not known exactly what effect each hormone exerts nor is it certain how these effects are brought about Experiments with certain animals which establish measurable acts of submis

present the hormone produces a pressor or a depressor response this is referred to as an amphibaric response

Gastrointestinal canal Serotonin causes an increase in peristalsis which is probably due to stimulation of postganglionic cholinergic fibres in the intestine

Respiratory system The hormone causes bronchial constriction and hyperpnoea If serotonin be injected into the right auricle it causes vasoconstriction of the pulmonary vessels and a rise in pulmonary arterial pressure

Kidney Serotonin causes inhibition of diuresis in the water loaded rat probably as the result of afferent glomerular arteriolar constriction

Central nervous system Injections of serotonin in normal volunteers may produce no subjective symptoms or may cause dyspnoea abdominal pain tingling prickling and a sense of impending death

DISTRIBUTION Serotonin is found in the alimentary canal in blood (where it is largely confined to platelets) the spleen and brain of vertebrates the skin of amphibia, the salivary glands of octopods and in certain venoms and poisons The observation that platelets readily absorb 5 HT has given rise to the suggestion that it is formed by the megakaryocytes or that the hormone is formed in the entero-chromaffin cells and conveyed to other parts of the body in the platelets

Although serotonin is found in the brain there exists a blood brain barrier towards the hormone suggesting that it may be synthesised locally This suggestion is supported by the fact that the enzymes required for its synthesis and others capable of bringing about its destruction have been found in the brain

FUNCTION The physiological significance of serotonin is unknown It does not seem likely that the hormone normally plays a part in renal function haemostasis or in the control of vasomotor tone Perhaps serotonin is important in the regulation of peristalsis

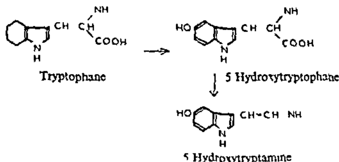
The most intriguing possibilities have been raised by the discovery of serotonin in the hypothalamus and other parts of the brain The hormone has been shown in vitro to cause contraction of oligodendroglial cells of the rat and of man Excess serotonin in the brain causes a number of complex disturbances of behaviour while a decrease in brain content of serotonin causes lethargy At present it is not possible to say more than that the hormone probably plays some part in maintaining normal mental processes

The technical difficulties involved in the study of behaviour are so great that the effects of various hormones have been very incompletely studied, but such evidence as exists favours the view that these effects may be far reaching

SEROTONIN

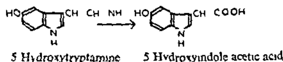
Among the hormones which have been studied because of their effect upon the central nervous system one of the most interesting is serotonin or 5 hydroxytryptamine (5 HT) This substance has been found in many normal tissues while certain tumours produce 5 HT in large quantities

SYNTHESIS Serotonin is formed from dietary tryptophane by preliminary hydroxylation to 5 hydroxytryptophane and subsequent decarboxylation



The first step is controlled by an enzyme system present in the liver while the decarboxylase concerned in the second step is widely distributed throughout the body

METABOLISM Serotonin is oxidised by amine oxidase in the lungs liver and kidney, to 5 hydroxyindole acetic acid (5HIAA) in which form it appears in the urine



ACTION Cardiovascular system Serotonin produces tachycardia and exerts a variable effect on blood pressure The last named effect results from the direct action of the hormone upon blood vessels sometimes serotonin causes vasoconstriction sometimes it abolishes existing neurogenic vasoconstriction causing vasodilatation According to the degree of neurogenic control

CHAPTER XIV

STEROID METABOLISM

In Chapters III IV and V the important steroid hormones have been discussed with some reference to their chemistry and their metabolism the present chapter is intended to amplify the chemi cal aspects of steroid metabolism Recent advances in the bio chemistry of steroids have given meaning to the bewildering number of isolated observations which have emerged from earlier experiments The fuller understanding of steroid metabolism result ing from this recent knowledge is necessary for the appreciation of present concepts of abnormal adrenocortical and testicular function

The ultimate aim of researches into the metabolism of steroid hormones is the complete elucidation of the number and chemistry of these hormones their precursors their metabolites and the steps involved in their synthesis and their catabolism So far this has not been achieved It is possible to isolate at least 29 steroids from extracts of the adrenal cortex of these some are physiologically inert while others are known to influence various physiological processes within the body Some of these twenty nine steroids are to be found in the blood of the adrenal vein while others have not been found outside the gland It is moreover important to consider the possibility that the processes by which these steroids are separ ated from biological fluids may affect their structure This is especi ally true of C_{9} steroids which in some instances could result from destruction of a side chain attached to the C of steroids contain ing 21 carbon atoms

The testis produces a number of C_{9} and C_{1} steroids some of which are androgens others are physiologically inert while at least one isolated from the testis of the boar appears to stimulate spermatogenesis

Following their entry into the blood stream *steroid hormones* exercise their biological functions and thereafter the body dis poses of these substances as follows —

(i) Small proportions of the hormones are excreted in urine in conjugated forms (page xiii)

(ii) Most of the steroid hormones undergo a series of reactions

REFERENCES

The subject matter of this chapter presents a brief and necessarily inadequate summary of the work of G W Harris which is described in greater detail in his book "Neural Control of the Pituitary Gland" Edward Arnold London 1955 This book gives full references to the literature on this subject.

1 Benedek T and Rubenstein B B Psychosomatic Med 1 245 1939
2 Benedek T and Rubenstein B B Psychosomatic Med 1 461 1939

CHAPTER XIV

STEROID METABOLISM

In Chapters III IV and V the important steroid hormones have been discussed with some reference to their chemistry and their metabolism the present chapter is intended to amplify the chemical aspects of steroid metabolism Recent advances in the biochemistry of steroids have given meaning to the bewildering number of isolated observations which have emerged from earlier experiments The fuller understanding of steroid metabolism resulting from this recent knowledge is necessary for the appreciation of present concepts of abnormal adrenocortical and testicular function

The ultimate aim of researches into the metabolism of steroid hormones is the complete elucidation of the number and chemistry of these hormones their precursors their metabolites and the steps involved in their synthesis and their catabolism So far this has not been achieved It is possible to isolate at least 29 steroids from extracts of the adrenal cortex of these some are physiologically inert while others are known to influence various physiological processes within the body Some of these twenty nine steroids are to be found in the blood of the adrenal vein while others have not been found outside the gland It is moreover important to consider the possibility that the processes by which these steroids are separated from biological fluids may affect their structure This is especially true of C_9 steroids which in some instances could result from destruction of a side chain attached to the C_1 of steroids containing 21 carbon atoms

The testis produces a number of C_{19} and C steroids some of which are androgens others are physiologically inert while at least one isolated from the testis of the boar appears to stimulate spermatogenesis

Following their entry into the blood stream steroid hormones exercise their biological functions and thereafter the body disposes of these substances as follows —

(i) Small proportions of the hormones are excreted in urine in conjugated forms (page xiii)

(ii) Most of the steroid hormones undergo a series of reactions

which alter their chemical structure In this altered form they are excreted in the urine The chemical changes involved leave the steroid nucleus intact with the result that the urinary metabolites take the form of conjugated steroids Some of these metabolites are physiologically inert others are active Some of these changes in the steroid hormones are brought about in the liver,

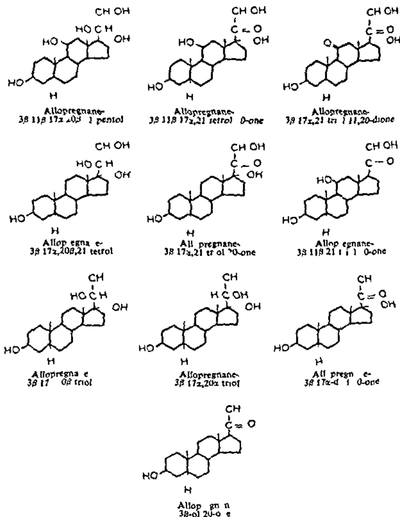


Fig 62 Adrenocortical steroids possessing the 3β 5α configuration It should be noted that these compounds have been isolated from beef adrenal tissue and that 5α 20β compounds are not commonly found in human urine The presence of these steroids in human adrenal tissue has yet to be confirmed

Cortico te one [C]

11-deoxycort cost [C]

11-dehydrocort co t n [C]

17 hyd xy 11-deo ycorti oste one [C]

17 hyd o y 11-d hyd ocort co te o e [Cort o] [C]

17 hydroxycort co t [Hyd ocortisone] [C]

Δ Pregn e-11β 21-diol 3 20-dione 18-al [Aldo te] [C]

Δ Pr gn 11β 17α,20β 21 tet l 3-o

Δ Pregne e-17α,20β 21 tri 1 3 11-d o e

Δ Pregn e-20β 21-d l 3 11-di ne

17 hyd yprog te n

Pr g st o

Fig 63 Adrenocortical steroids with Δ 3 ketone configuration

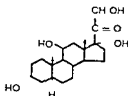


Fig 64 Allopregnane-3 11β 17α 21 tetrol 20-one

the site of other changes is unknown Much still remains to be learnt about the renal clearance of steroids while the subject of their faecal excretion has been studied only within recent years

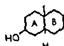
Synthesis

The steps involved in the synthesis of steroid hormones are not known in detail It seems likely that such hormones are derived from cholesterol while cholesterol in turn is built up from acetate—that is from the active 2 carbon atom fragments which unite with coenzyme A to give acetyl coenzyme A (see page 204) The synthesis of adrenocortical steroids is discussed in Chapter III

ADRENOCORTICAL STEROIDS

It has been pointed out that twenty nine steroids have been isolated from the adrenal cortex These can be classified as follows —

1 Ten steroids have the 3β 5α configuration i e



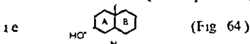
These are shown in Fig 62 No biological activity has been shown by these compounds

2 Twelve steroids show a Δ^4 3 ketone i e



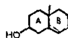
These are shown in Fig 63 Seven of these exhibit corticosteroid activity (either glucocorticoid or mineralocorticoid or both) these are indicated by [C] in Fig 63 Progesterone is a member of this group and is the most active progestational hormone occurring in nature

3 One steroid is unique in possessing 3α, 5α configuration



4 Three steroids are derivatives of androstane these are shown in Fig 65 together with oestrone

5 Dehydroepiandrosterone* is a Δ^5 compound i e



(Fig 66)

* Most authors now prefer to use the name dehydroisoandrosterone instead of dehydroepiandrosterone

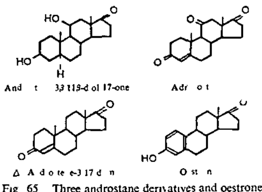


Fig 65 Three androstane derivatives and oestrone

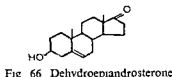


Fig 66 Dehydroepiandrosterone

TESTICULAR STEROIDS

The most important steroids to be found in testicular tissue are those which contain 19 carbon atoms namely —

(i) Testosterone (Δ^4 androsten 17β ol 3-one)

(ii) Δ^4 Androstene 3 17 dione

(iii) Δ^{16} Androsten 3α-ol

(iv) Δ^{16} Androsten 3β ol

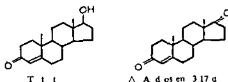


Fig 67 Testicular steroids

Of these the first two have been isolated from perfusion studies in which isotopically labelled acetate and a gonadotrophic hormone preparation were perfused through the human testis These hormones show typical androgenic activity The remaining two compounds have not so far been found in the human testis and they have not yet been shown to possess androgenic activity (Fig 67) Three C_{1} steroids have been isolated from the testis of the boar but these compounds are not androgens

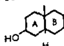
the site of other changes is unknown Much still remains to be learnt about the renal clearance of steroids while the subject of their faecal excretion has been studied only within recent years

Synthesis

The steps involved in the synthesis of steroid hormones are not known in detail It seems likely that such hormones are derived from cholesterol while cholesterol in turn is built up from acetate—that is from the active 2 carbon atom fragments which unite with coenzyme A to give acetyl coenzyme A (see page 204) The synthesis of adrenocortical steroids is discussed in Chapter III

ADRENOCORTICAL STEROIDS

It has been pointed out that twenty nine steroids have been isolated from the adrenal cortex These can be classified as follows —

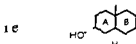
1 Ten steroids have the 3β 5α configuration i e 

These are shown in Fig 62 No biological activity has been shown by these compounds

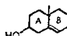
2 Twelve steroids show a Δ^4 3 ketone i e 

These are shown in Fig 63 Seven of these exhibit corticosteroid activity (either glucocorticoid or mineralocorticoid or both) these are indicated by [C] in Fig 63 Progesterone is a member of this group and is the most active progestational hormone occurring in nature

3 One steroid is unique in possessing 3α 5α configuration

i e  (Fig 64)

4 Three steroids are derivatives of androstane these are shown in Fig 65 together with oestrone

5 Dehydroepiandrosterone* is a Δ^5 compound i e 

(Fig 66)

* Most authors now prefer to use the name dehydroisoandrosterone instead of dehydroepiandrosterone

1 *Androsterone and two isomers* Figure 68 shows three 17-ketosteroids which are found in normal urine They are stereoisomers differing only in the position of the OH group at C_3 and that of the H at C_5 The third of these isomers aetiocholan 3α ol 17 one is not an androgen All three of these steroids lack an oxygen at C_{11}

2 11 *oxygenated steroids* This group consists of the 12 steroids in Fig 69 Of these only four are 17 ketosteroids and only one (androstane 3α 11β diol 17 one) is known to possess androgenic properties It should be pointed out however that the 21 carbon atom members of this group may yield androgens and/or 17 ketosteroids on removal of their side chains

3 Δ^4 3 *ketosteroids* It has recently become possible to isolate steroids containing the group [A] from the urine Apart from cortisone and hydrocortisone which are to be found in normal urine a number of steroids with this grouping have been isolated from the urine of patients suffering from diseases of the adrenal cortex

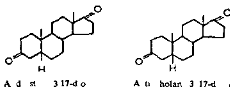


Fig 70 Two saturated C diones found in urine

4 *C ₉ saturated diones* Figure 70 shows two saturated diones which have been isolated from normal urine

5 Δ^5 *steroids* Normal human urine contains a number of steroids which possess a double bond between C and C_6 Two of these are shown in Fig 71

6 *Pregnane* 3α 17α 20α *triol* which is shown in Fig 72

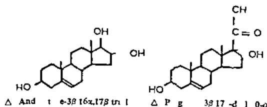


Fig 71 Urinary steroids with Δ structure
Dehydroepiandrosterone also shows this structure

THE STEROIDS OF URINE

Among the steroid compounds to be found in urine the most important are those which are associated with the metabolism of steroid hormones

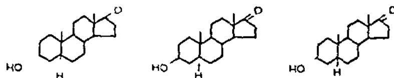
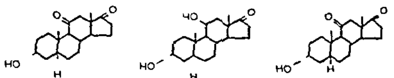
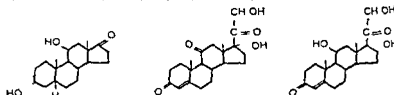
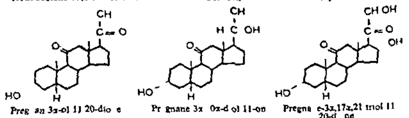


Fig 68 Urinary steroids Androsterone and two isomers







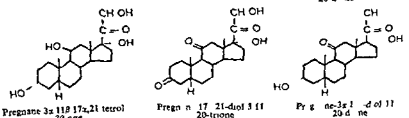


Fig 69 11-oxygenated urinary steroids

* The prefix aetio- is spelt etio by American authors

(b) Those which show a 20 ketone but not a 17 hydroxy group do not give rise to 17 ketosteroids —

Corticosterone

11 dehydrocorticosterone

11-deoxycorticosterone

Progesterone

Aldosterone

3 The unique steroid allopregnane 3α 11β 17α 21 tetrol 20-one gives rise to 17 ketosteroid metabolites

4 The three androgens derived from androstane (Fig 65) give rise to 17 ketosteroid metabolites

5 Dehydroepiandrosterone also contributes to the 17 ketosteroids of the body

Hydrocortisone cortisone adrenosterone and Δ^4 androsten 11β ol 3 17 dione are metabolised to the same four ketosteroids Hydrocortisone and cortisone give rise to more aetiocholane derivatives than to androsterones while the opposite is the case with adrenosterone and Δ^4 androsten 11β ol 3 17 dione (Fig 73)

In addition cortisone and hydrocortisone are metabolised to the following steroids of which the first two are the major metabolites of cortisone and hydrocortisone respectively —

(i) Pregnane 3α 17α 21 triol 11,20 dione (tetrahydrocortisone or tetrahydro E)

(ii) Pregnane 3α 11β 17α 21 tetrol 20 one (tetrahydrohydrocortisone or tetrahydro F)

(iii) Pregnane 17α 21 diol 3 11 20 trione

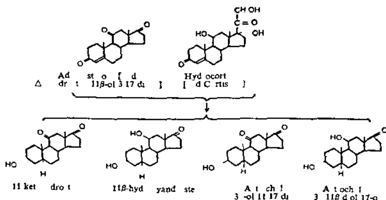


Fig 73 The metabolism of both adrenosterone and hydrocortisone to the same four 17 ketosteroids

7 *Miscellaneous steroids* In addition to the important steroids of normal human urine which have been mentioned a number of steroids are found in the urine of patients suffering from endocrine diseases and others have been isolated from the urine of other species

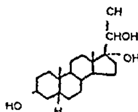


Fig 72 Pregnane 3α 17α 20α triol

THE RELATIONSHIP BETWEEN THE STEROIDS OF THE BODY AND THEIR EXCRETORY PRODUCTS

Some of the steroid hormones of the body are excreted unchanged some are converted to C_{19} steroids (i e to 17 ketosteroids) and excreted in this form some are excreted as C_{21} steroids (with or without a hydroxyl group at C_{17}) Although the metabolic fate of every steroid hormone is not known it is possible to indicate the relationship between the important urinary steroids and their precursors within the body

METABOLISM OF ADRENOCORTICAL STEROIDS Some of the steroids found in adrenocortical extracts give rise to 17 ketosteroid metabolites others do not The relationship between adrenocortical steroids and urinary 17 ketosteroids may be indicated as follows —

1 Very little information is available concerning the fate of the ten steroids which share 3β 5α configuration These compounds are not biologically active and the elucidation of their significance and their metabolism awaits further experimental studies

2 Among the group of steroids with a Δ^4 3 ketone group some give rise to 17 ketosteroid metabolites and others do not

(a) Those which show 17 hydroxy and 20 ketone groups do give rise to 17 ketosteroids —

Hydrocortisone*

Cortisone

17 hydroxy 11 deoxycorticosterone

17α hydroxyprogesterone

* Hydrocortisone is also known as cortisol

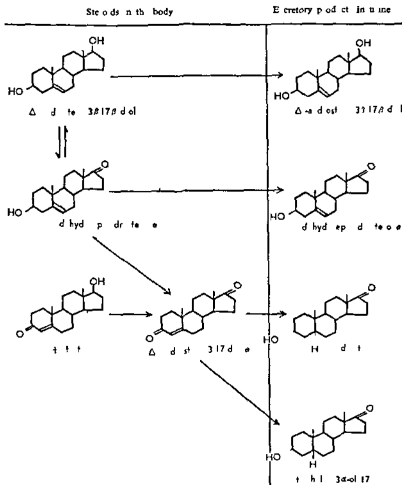


Fig 75 Common metabolic pathways in the metabolism of testosterone and dehydroepiandrosterone

METABOLISM OF TESTICULAR STEROIDS Testosterone is metabolised to the three stereoisomers androsterone epiandro sterone and aetiocholan 3α ol 17 one These are the chief metabolites of testosterone others include aetiocholane 3α 17β diol aetiocholane 3 17 dione androstane 3 17 dione and possibly androstane 3α 17β diol These metabolites are shown in Fig 76 from which it can be seen that the three stereoisomers and two other metabolites are 17 ketosteroids

17 hydroxy 11-deoxycorticosterone and 17α hydroxyprogesterone together with Δ^4 androstene-3,17-dione and dehydroepiandrosterone are metabolised to androsterone and to aetiocholan 3α-ol 17-one (Fig 74) The two C_{19} steroids give rise to much more aetiocholanolone than androsterone, while dehydroepiandrosterone can also give rise to three other steroids (Fig 75)

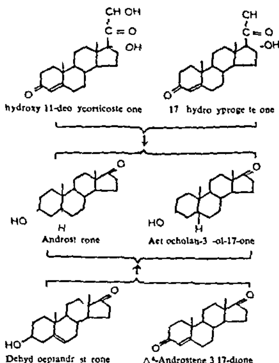


Fig 74 The metabolism of two 17 hydroxy and of two 17 ketosteroids

11 deoxycorticosterone and progesterone are both converted to pregnane 3α 20α diol The metabolism of aldosterone is still uncertain but some of this hormone is excreted unchanged

The metabolic fate of androstane 3β 11β diol 17 one is uncertain but it seems probable that this steroid is converted to 11β hydroxyandro terone and to 11 ketoandrosterone

The unique steroid allopregnane 3α 11β 17α,21 tetrol 20-one may give rise to androstan 3α-ol-11 17 dione

The metabolism of dehydroepiandrosterone is shown in Fig 75 It will be seen that this compound gives rise to two 17 ketosteroids and to a 17 hydroxy compound

4 The two 11-oxygenated androsterone derivatives (i e 11-ketoandrosterone and 11β hydroxyandrosterone) are almost entirely formed from 11-oxygenated $\Delta^4$3 17 ketosteroids (i e adrenosterone and Δ^4 androsten 11β-ol 3 17-dione) with very small contributions from cortisone and hydrocortisone

5 The two 11-oxygenated aetiocholane derivatives (i e aetiocholan 3α-ol-11 17-dione and aetiocholane 3α 11β diol-17 one) are largely derived from cortisone and hydrocortisone with negligible contributions from adrenosterone and Δ^4 androsten 11β-ol 3 17-dione

6 The appearance of cortisone and hydrocortisone in normal human urine is taken to indicate that some of these two steroids passes into the urine unchanged

7 The two saturated C_{19} diones (i e aetiocholane 3 17-dione and androstane 3 17-dione) are derived from the metabolism of testosterone

8 The Δ^5 steroids Dehydroepiandrosterone appears in the urine and represents the excretion of a steroid hormone unchanged while Δ^5 androstene 3β 16α 17β triol and Δ^5 androstene 3β 17β diol are derived from dehydroepiandrosterone

9 Pregnane 3α 17α 20α triol arises from the metabolism of 17α hydroxyprogesterone

THE ESTIMATION OF URINARY STEROIDS

ANDROGENS AND 17 *KETOSTEROIDS* A number of androgenic steroids are excreted in normal urine These are either metabolites of adrenocortical and testicular steroids or androgenic hormones excreted unchanged Most of these androgens possess a ketone group at C_{17} but it should be noticed that not all 17 ketosteroids of urine are androgens (e g aetiocholan 3α-ol 17-one) and that some urinary 17 ketosteroids are derived in part from non androgenic precursors (e g cortisone gives rise to aetiocholan 3α-ol 11 17 dione) Methods of bioassay measure only androgenic steroids while chemical methods of total 17 ketosteroid estimation measure all steroids with a ketone group at C_{17} regardless of whether these are androgenic or not on the other hand such methods will not measure androgens which are not 17-ketosteroids Nevertheless chemical assay of 17 ketosteroids and bioassay of androgens show a correlation which is of such an order as to encourage the use of the simpler chemical technique for everyday purposes at the expense of bioassay which is reserved for special studies

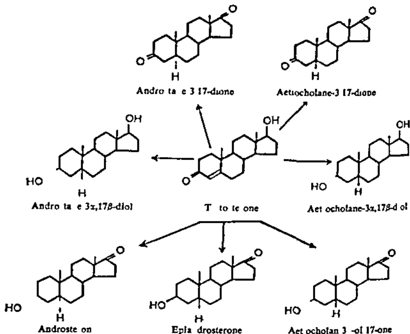


Fig 76 The metabolism of testosterone

Figure 75 shows the metabolism of testosterone and dehydroepiandrosterone through the common metabolite Δ^4-androstene 3, 17-dione which in turn gives rise to androsterone and aetiocholan 3α-ol-17-one

To view this relationship from the other end of the metabolic scale we can trace the origin of the important urinary 17 ketosteroids from their precursors

1 Androsterone is derived chiefly from Δ^4 androstene 3 17 dione and hence indirectly from testosterone and dehydroepiandrosterone with small contributions from 17α hydroxyprogesterone and 17α hydroxy-11-deoxycorticosterone

2 Aetiocholan 3α ol 17-one arises from the following sources

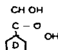
(i) Testosterone
(ii) Dehydroepiandrosterone
(iii) Δ^4-Androstene 3,17 dione
(iv) 17α hydroxyprogesterone
(v) 17α hydroxy 11 deoxycorticosterone

3 Epiandrosterone arises from the metabolism of testosterone

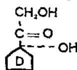
such a small part of the β fraction that it can be ignored The β fraction can be precipitated by digitonin and hence measured separately or dehydroepiandrosterone itself can be subjected to chemical assay In the urine of normal adults the β fraction constitutes about 15 per cent of the total neutral 17 ketosteroids

17 *HYDROXYCORTICOSTEROIDS* It was pointed out on page 47 that a number of methods have been elaborated for the estimation of urinary steroids which bear a hydroxy group at C_{17} Of these methods two were mentioned namely —

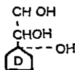
(1) The Porter-Silber method which measures steroids possessing the following group —



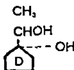
(2) Oxidation methods which measure steroids possessing the following groups —



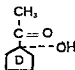
(a)



(b)



(c)



(d)

A number of oxidation methods have been devised and certain of these procedures measure steroids with some of the groups shown in (a) to (d) For example the method of Norymberski can be used to measure (a) (b) (c) or by adding another step to the procedure (a) (b) (c) and (d)

Among the known urinary steroids the following are measured by the Porter Silber method

(i) Pregnane 3α 17α 21 triol 11 20-dione
(ii) Pregnane 3α 11β 17α 21 tetrol 20 one
(iii) Pregnane 17α 21-diol 3 11 20 trione
(iv) Pregnane 3α,17α diol 11 20 dione
(v) Δ^5 Pregnene 3β 17α diol 20 one

It will be seen from the preceding pages of this chapter that (i) (ii) and (iii) are products of the metabolism of the glucocorticoids cortisone and hydrocortisone The oxidation method will measure all these compounds and in addition Pregnane 3α,17α 20α triol together with a number of steroids the exact composition of which has not been determined It is therefore clear that both methods provide a means of estimating products of glucocorticoid metabolism but that the oxidation method will provide higher

DETERMINATION OF 17-KETOSTEROIDS The most widely used method for the determination of 17 ketosteroids in volves the colour produced by reaction with *m*-*dinitrobenzene* This is called the Zimmerman reaction and can be used to esti mate all 17-ketosteroids or neutral 17 ketosteroids the neutral fraction excludes those 17 ketosteroids in which the A ring is phenolic  e g oestrone Table I shows the normal range of total 17-ketosteroid excretion for various age groups

TABLE I*

Age years	*17 ketosteroids mgm /day*	
	Male	*Female*
3-4	2 4-3 0	1 2 2 7
9 10	7 8-8 2	5 0
14-15	6 9 15 9	6 2 11 6
20-40	11 6 34 0	4-22
Over 60	2 8 12 5	1 6-6 0

*After R I Dorfman (1957)

THE β FRACTION OF 17 *KETOSTEROIDS* A number of 17-ketosteroids possess a hydroxyl group at C_3 In most of these compounds the OH occupies the α position in relation to the methyl group at C_{10} (see page 8) but in certain *steroids it occupies the β position β 17 ketosteroids are for the most part* secreted by the adrenal cortex estimation of the β fraction of 17-ketosteroids therefore provides more specific information about cortical activity by eliminating 17 ketosteroids derived from testi cular androgens Among the known urinary 17 ketosteroids the *following possess this β configuration*

(i) Dehydroepiandrosterone

(ii) Δ^5 Androstene 3β 16α 17β triol

(iii) Δ^5 Androstene 3β 17β diol

(iv) Epiandrosterone

Dehydroepiandrosterone constitutes the bulk of the β fraction of urinary 17 ketosteroids so that for all practical purposes it is safe to regard this fraction as adrenocortical in origin Epiandrosterone is a metabolite of testosterone (Fig 75) but constitutes

3 *Metabolism of C_{21} steroids to 17 ketosteroids* There is good reason to believe that the conversion of C_{21} steroids with a Δ^4 3-ketone group involves the reduction of the double bond in the A ring before the side chain at C_{17} is removed when these steroids are being metabolised to 17 ketosteroids This is shown by the fact that C_{21} compounds with a Δ^4 3 ketone group yield much greater quantities of 5β isomers than of 5α. If the side chain were first removed, the oxygen at C_{11} which most of these compounds contain would direct the reduction in ring A in favour of 5α isomers (see 1 above)

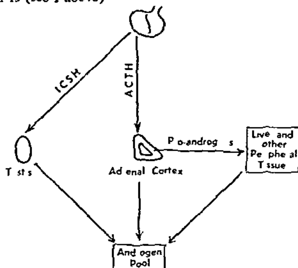


Fig. 77 Diagrammatic representation of the control of androgen secretion.

CONTROL OF ANDROGEN SECRETION

Although the adrenal cortex contributes about twice as much as the testis towards the total urinary 17 ketosteroids adrenocortical androgens are weaker in their physiological effects than the androgens of the testis In addition to secreting androgens the cortex secretes a number of steroids which are converted in part to androgens These steroids are called pro-androgens and include cortisone hydrocortisone 17-hydroxyprogesterone and 17α hydroxy 11-deoxycorticosterone

Among the factors which control the rate of secretion of androgens two trophic hormones of the adenohypophysis are important (Fig 77) It is generally believed that these trophic hormones operate by means of their influence upon enzyme systems

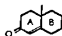
figures than the Porter-Silber method because the former measures a number of compounds which are not estimated by the latter

It can therefore be seen that while the total neutral 17 ketosteroid level in urine provides some measure of the rate of secretion of androgens in the body the 17 hydroxysteroids reflect the rate of secretion of glucocorticoids The β fraction of 17 ketosteroids is a useful guide to the adrenal contribution to the total 17-ketosteroid output It has become possible to estimate the concentration of aldosterone in urine but so far this has been reserved for special studies because the amount of this substance present in normal human urine is very small (3-4 μgm per 24 hours) which makes its estimation difficult

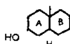
GENERAL METABOLIC PATHWAYS

During the performance of the experiments which have provided the data upon which this chapter is based certain chemical features of the steroid hormones were found to influence the metabolic path ways these substances followed Some of these influences can be indicated in general terms

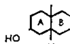
1 *Reduction of Δ^4 3 ketone in C_{19} steroids* When the double bond at C_4 is reduced in steroids which show the following grouping



the hydrogen added to C_5 will adopt the α or β configuration in roughly equal amounts, i e

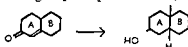


and



However when the steroid concerned bears an oxygen at C_{11} the product of this reduction is predominantly α At the same time it is interesting to notice that during the reduction of the 11 oxygenated steroids of this type the oxygen is not removed (i e no 11 deoxy C_{19} metabolites are formed) but most of the 11 ketone groups are reduced to the 11β hydroxy grouping

2 *Metabolism of C_{21} steroids to C_{21} metabolites* C_{21} steroids are metabolised either to C_{21} products or to C_{19} compounds It has been found that the presence of a *side chain at C_{17} regardless* of the presence or absence of an oxygen at C_{11} causes the reduction of a Δ^4 3 ketone group to produce a 5β configuration i e



INDEX

TABLE II
Relative activity of Androgens on Capon s Comb
(After Dorfman and Shipley)

Compound	*Weight of Compound (ug) equivalent to 100 ug of Androsterone (1 I U)*
Testosterone Androstan 17β-ol 3-one Androstane 3α 17β diol Methyltestosterone	15-30
Δ^5 Androstene 3β 17β diol	30-60
Androsterone Δ^4 Androsten 3α ol 17-one Δ^4 Androstene 3 17 dione Androstane 3 17 dione	60-120
Dehydroepiandrosterone	120-250
Δ^5 Androstene 3 17 dione Δ^5 Androstene 3α 17β diol	250 500
Epiandrosterone Δ^5 Androstene 3β 17α diol	500 1000
Aetiocholan 3α ol 17 one Aetiocholane 3α 11β diol 17-one	>1000 or inactive

RELATIVE ACTIVITIES OF ANDROGENS Table II shows the comparative biological activity of a number of steroids on the basis of their effect upon the comb of the capon This table illustrates the wide range of potency of various steroids but the relative potencies are only approximate because rigid statistical comparison has not been undertaken The relative potencies vary from one test to another and according to the mode of administration of the steroids, whether local or systemic

REFERENCES

The material upon which this chapter is based comes from Androgens by Dorfman R I and Shipley R A Wiley New York 1957 This book contains a comprehensive survey of the chemistry and physiology of androgens and related steroids in health and disease together with full references to the literature

INDEX